University of Alberta

SYNERGISTIC EFFECT DURING THE SEQUENTIAL INACTIVATION OF *CRYPTOSPORIDIUM* OOCYSTS IN NATURAL WATERS

By

Kaushik Biswas

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled SYNERGISTIC EFFECT DURING THE SEQUENTIAL INACTIVATION OF *CRYPTOSPORIDIUM* OOCYSTS IN NATURAL WATERS submitted by KAUSHIK BISWAS in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL ENGINEERING

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DEDICATION

I dedicate this thesis to my parents, my sister, and my wife, Sanchita, for their love, support, and inspiration throughout this work.

ABSTRACT

Cryptosporidium parvum oocysts are known to be a frequent cause of waterborne diseases. Earlier studies have shown that sequential treatment of *C. parvum* oocysts with oxidants in buffered de-ionized water can lead to synergistic inactivation of parasites. The purpose of this study was to provide a detailed evaluation of the synergistic effect of *C. parvum* inactivation in natural waters instead of buffered de-ionized water.

It was found that, for the range of experimental conditions investigated, the magnitude of the synergistic effect of sequential treatment of *C. parvum* with ozone followed by free chlorine in five high pH (8) natural waters was considerably lower than previously reported for buffered de-ionized water. However, in the two low pH (6) natural waters, the magnitude of the synergistic effect was comparable to that previously reported for buffered de-ionized water. It was concluded that the inhibition of synergistic effect in the high pH natural waters tested was in part due to alkalinity or pH and in part due to other unidentified water quality characteristics.

For ozone followed by monochloramine sequential treatment, the magnitude of the synergistic effect measured in the natural waters was comparable to that previously reported for buffered de-ionized water for the conditions studied. Statistically significant impacts of ozone pre-treatment level, pH, and water quality were observed on the synergistic effect. For those water treatment plants able to provide sufficient contact times, ozone followed by monochloramine may be a practical means of achieving additional *C. parvum* inactivation credit due to the synergistic effect.

For the inactivation of *C. parvum* oocysts using ozone only, a linear Chick-Watson model was found to be adequate for describing the inactivation kinetics in the studied natural waters. The results with the Chick-Watson model indicated a statistically significant effect of temperature, batch-to-batch variation of the oocysts resistance to ozone, and the oocysts age on the inactivation kinetics of *C. parvum* after treatment with ozone. The individual water quality parameters, including pH, did not affect the inactivation kinetics significantly. Based on the Chick-Watson model, ozone disinfection design criteria for the inactivation of *C. parvum* in natural water were established.

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LIST OF ABBREVIATIONS, UNITS, AND SYMBOLS

a	empirical constant
AWWA	American Water Works Association
AwwaRF	American Water Works Association Research Foundation
b	empirical constant
B	average of all the trials of the ozone inactivation of the oocysts per unit ozone Ct for a particular batch and temperature [log/(mg·min/L)]
c	empirical constant
°C	degrees Celsius
C	oxidant concentration at time t, (mg/L)
Ct	product of the concentration and contact time (mg·min/L)
C_{avg}	geometric average of C_0 and C_f, (mg/L)
C_f	oxidant residual measured at the end of the applied contact time, (mg/L)
C_0	oxidant concentration measured at time zero, (mg/L)
$Cl_2(aq)$	free chlorine (aqueous)
$Cl_2(g)$	free chlorine (gas)
$Ct_{1\text{-log}}$	Ct required for 1 log-unit synergistic effect, (mg·min/L)
CI	statistical confidence interval
d	estimated infectious oocysts in the inoculum of each mouse, (No./mouse)
d_0	total number of oocysts in each inoculum as counted by hemacytometer, (No./mouse)
DBPs	disinfection by products
DI	deionized
DPD	(*N,N*-diethyl-p-phenylenediamine)

FBRR	Filter Backwash Recycling Rule (U.S. EPA)
GWUDI	Groundwater Under Direct Influence (U.S. EPA)
H_2O	water
HOCl	hypochlorous acid
ICR	Information Collection Rule
IESWTR	Interim Enhanced Surface Water Treatment Rule (U.S. EPA)
ID_{50}	estimated oocyst dose for 50% of infection of the CD-1 neonatal mice in a cohort
k	first-order Chick-Watson rate constant, (min^{-1})
k'	inactivation rate constant for linear relationship
k''	inactivation rate constant for a particular microorganism
k_d	first-order chemical disinfectant disappearance rate constant, (min^{-1})
k_1	inactivation rate constant for secondary treatment alone, (min^{-1})
k_2	inactivation rate constant for secondary treatment following ozone primary treatment, (min^{-1})
L	likelihood function for normally distributed errors
L	litre (in unit)
LT1ESWTR	Long Term 1 Enhanced Surface Water Treatment Rule (U.S. EPA)
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule (U.S. EPA)
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
m	empirical kinetic model parameter

mg/L	milligram per litre
mg·min/L	units of *Ct* product
min	minute
N	number of surviving oocysts at time t
N_0	number of oocysts at time zero
n	empirical kinetic model parameter
na	not available
NH_4^+	ammonium ion
NH_2Cl	monochloramine
$NHCl_2$	dichloramine
NCl_3	trichloramine
O_3	ozone
OCl^-	hypochlorite ion
P	proportion of mice in a cohort scoring positive for a given inoculum
SWTR	Surface Water Treatment Rule (U.S. EPA)
T	temperature (°C)
TCR	Total Coliform Rule (U.S. EPA)
TOC	total organic carbon (mg/L)
t	contact time (min)
U.S. EPA	United States Environmental Protection Agency
X	oocyst inoculum (log-units) given to each neonate CD-1 mouse

X_1	regression model input representing ozone primary treatment level (log-units)
X_2	regression model input representing temperature (°C)
X_3	regression model input representing pH
X_4	regression model input representing water quality
y_i	observed inactivation for the i^{th} trial
y	measured inactivation (log-units)
Y_i	positive ($Y_i 1$) or negative ($Y_i 0$) infection of the mice
Y	regression model output representing the magnitude of the synergistic effect $[(k_2-k_1)\times 10^4]$
$\hat{Y}$	I.g.H model prediction (log-units)
γ	incomplete gamma function
α	level of statistical significance
β	model parameters: for the logistic response model, refer to β_o and β_1
$\hat{\beta}$	optimal model prediction of β
θ	empirical constant
µg/mL	microgram per millilitre

CHAPTER 1: INTRODUCTION

1.1 CHALLENGES IN WATER TREATMENT

In the earlier part of the twentieth century the introduction of chlorination and filtration in drinking water treatment saved millions of lives throughout the world. Before chlorination the outbreaks of waterborne diseases like cholera and typhoid were quite common. Thus the prevention of outbreaks due to pathogenic microorganisms in public drinking water is considered to be one of the most significant achievements of the last century. In North America, chlorination was regarded as the most effective option against all pathogenic microorganisms both in terms of cost and treatment. For several years few questions were asked about the efficacy of chlorination and hence there was little room for improvement or alternative investigations. However, during the latter part of the twentieth century the water treatment industry was challenged by more resistant pathogens like *Giardia lamblia* and *Cryptosporidium parvum*. Several disease outbreaks were reported due to these waterborne pathogens (Smith and Rose 1998; Fayer et al. 2000; MacKenzie et al. 1994). This prompted the exploration of alternatives such as ozone, monochloramine, chlorine dioxide and UV radiation. At the same time, people became more and more aware and concerned about the potential long-term human health effects due to ingestion of chemical byproducts formed during chlorination or other chemical treatment processes. All these factors led to new challenges for the water treatment industry to come up with a novel and cost effective solution for striking a balance between the risk of microbial contamination and the long-term health effects due to chemical byproducts.

Today the microorganism reduction approach in water treatment is evolving in response to the need to more rigorously protect public health.

1.2 SEQUENTIAL TREATMENT FOR MICROORGANISM REDUCTION

Ingestion of *Cryptosporidium* spp. oocysts can cause the disease cryptosporidiosis, a potentially life-threatening diarrheal illness in persons with underdeveloped or suppressed immune systems (Colford et al. 1996; Gerba et al. 1996). Water suppliers for a long time have been concerned that conventional treatment methods may not be a sufficient barrier to waterborne transmission of cryptosporidiosis. The enhanced surface water treatment rule (ESWTR) under development in the United States will include requirements for *Cryptosporidium parvum* inactivation. The long contact time (t) and high chemical dose (C) needed to achieve Ct values required for *C. parvum* inactivation raise the issues of chemical by-products formation and feasibility as well as cost of compliance with future regulations. Free and combined chlorine have limited capabilities to inactivate *C. parvum* (Korich et al 1990, Gyürék et al. 1997). Ozone and chlorine dioxide can effectively inactivate *C. parvum* (Finch et al. 1994, Korich et al. 1990). However, the levels of ozone or chlorine dioxide required to achieve oocyst inactivation may lead to problems with chemical byproducts formation.

Synergism is the cooperative interaction of two or more substances, or phenomena, producing a greater total effect than the sum of their individual effects (University of Wisconsin-Stevens Point). Studies at the University of Alberta and elsewhere have shown that ozone primary treatment followed by either chlorine or chloramines secondary treatment can achieve levels of inactivation that are greater than those predicted based on simple addition of the inactivations expected for each chemical alone (Li et al 2001 a, b; Driedger et al 1999 a, b). Thus there appears to be a synergistic effect of the sequential treatment on inactivation of the oocysts. The limitation of these studies was that they were conducted exclusively in prepared laboratory buffers and may not necessarily be representative of inactivation of oocysts in naturally occurring waters. The U.S. EPA is developing Ct tables for inactivation of *Cryptosporidium* spp., and there is a need to include, if confirmed, the synergistic effect of sequential treatment. A previous study (Oppenheimer et al., 2000) of *Cryptosporidium* spp. inactivation in natural waters suggested that initial microorganism reduction with ozone can enhance the

inactivation achieved by secondary exposure to chlorine in natural waters. The authors of that report, however, concluded that additional data are required to more fully define water quality effects.

1.3 RESEARCH OBJECTIVES

The purpose of this study was to provide a more detailed evaluation of the synergistic effect during sequential treatment of *Cryptosporidium* spp. oocysts in natural waters. The specific research objectives were:

- To quantify the synergistic effect of ozone primary treatment followed by either chlorine or monochloramine secondary treatment in various natural surface waters.
- To determine which of the following experimental factors have a significant impact on the synergistic effect:
    1. the type of secondary treatment (free chlorine or monochloramine);
    2. the level of primary ozone treatment;
    3. the pH of the water;
    4. the temperature of the water; and
    5. the quality of the water.
- To compare the synergistic effect measured in the natural waters to that observed in prepared laboratory buffers (Li et al 2001b).
- To determine the ozone inactivation kinetics of *C. parvum* in natural waters and establish an ozone inactivation design criteria of *C. parvum* in natural waters.

CHAPTER 2:LITERATURE REVIEW

2.1 BACKGROUND OF *CRYPTOSPORIDIUM PARVUM*

2.1.1 *Cryptosporidium parvum*

Cryptosporidium spp. are unicellular parasitic microorganisms known to infect a variety of vertebrate animals. *C. parvum* is the species that causes the gastrointestinal disease in humans known as cryptosporidiosis. E.E. Tyzzer in 1912 (Current and Garcia 1991) first described the life cycle stages of *C. parvum* in the gut of laboratory mice. However, for 48 years it remained relatively obscure, as it appeared to be of no economic, medical or veterinary importance. In 1976 the first case of cryptosporidiosis in humans was reported (Nime et al. 1976) and in 1985 the first documented outbreak of human cryptosporidiosis due to contamination of water supply was reported (D'Antonio et al. 1985). In 1993, interest in *C. parvum* expanded dramatically following a massive waterborne outbreak in Milwaukee, WI, involving an estimated 403, 000 persons (Fayer et al. 2000). Following that incident the general public, public health agencies, agricultural agencies and groups, environmental engineers and agencies, suppliers of drinking water, and others expressed concern and initiated studies on *C. parvum* with emphasis on developing methods for detection, prevention and treatment.

2.1.2 General Biology

Cryptosporidium spp. are intercellular protozoan parasites. The sporozoite of the parasite has specialized organelles, which assist the parasite in host cell penetration. These include rhoptries (electron-dense, tubular paired structures which are often posteriorly enlarged) and small elongate electron-dense micronemes (convoluted tubules, rod-shaped granules), which extend longitudinally in the anterior part of the body and may be attached to the rhoptries (Crawford and Vermund 1988). The species causing disease in humans is *C. parvum. C. parvum* appears to lack host specificity and has been

shown to cross-infect 79 different mammalian species, (O'Donoghue 1995; Tzipori and Griffiths 1998) including rodents, ruminants, dogs and humans.

Human and calf *Cryptosporidium* spp. isolates studied in mice, chick embryos, and cell cultures have six stages in the life cycle (Crawford and Vermund 1988). They are as follows (Figure 2.1):

Excystation: Excystation is the first stage, which involves the escape of four infective sporozoites through a sutural junction in the oocyst wall, invasion of the epithelial cells of the gastrointestinal tract and formation of trophozoites.

Merogony: The second stage in the life cycle is merogony (asexual multiplication) initiated after the sporozoites attach themselves to the surface of the host epithelial cell. During merogony, the trophozoite or type I meront undergoes nuclear divisions producing either six or eight nuclei. The nuclei migrate to the periphery of the parasite and form type I merozoites. The type I merozoites appear randomly arranged in a spherical mass within the parasitophorous vacuole, from which the type I meront releases six or eight type I merozoites. These merozoites may attach themselves to uninfected microvilli and repeat the asexual cycle, yet another means of autoinfection, or may form type II meronts.

Gametogony: The third stage, gamete formation, occurs when type I merozoites undergo secondary merogony, becoming type II meronts. Each type II meront forms four type II merozoites arranged longitudinally, with the anterior ends of all four merozoites directed away from the feeder organelle. These type II merozoites, which are shorter and broader than the type I merozoites, are released by rupture of the parasitophorous vacuole and penetrate the microvillous border of intestinal epithelial cells.

Fertilization: During the fourth stage of the life cycle, fertilization, the microgametes attach to the host cell membrane covering the macrogamete. Upon penetration, the diploid oocyst is formed.

Oocyst wall formation: The fifth stage is oocyst wall formation. The outer oocyst wall is made by type I wall-forming bodies and the inner wall, by type II wall-forming bodies.

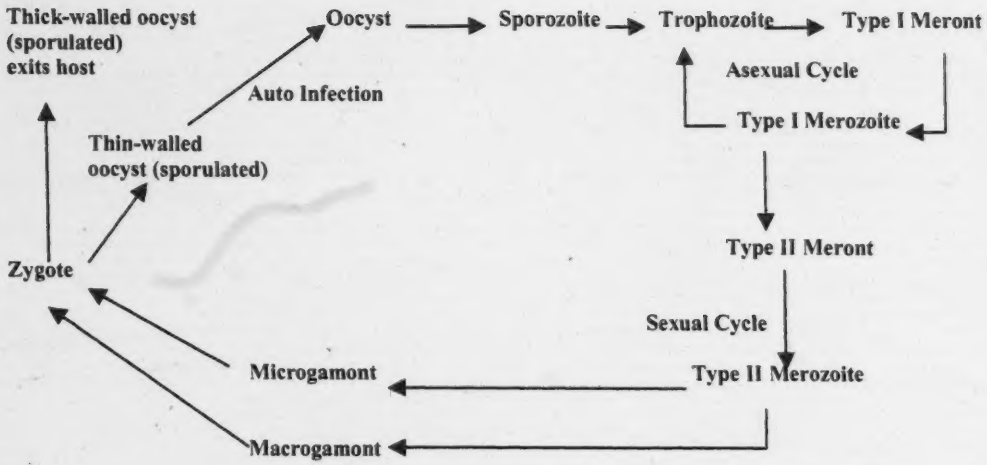

Figure 2.1 Schematic layout of *Cryptosporidium parvum* life cycle

Sporogony: The sixth stage is sporogony of the oocyst, which occurs within the parasitophorous vacuole. The sporont separates from the oocyst residium and divides into four sporoblasts, which become the long slender sporozoites. Sporozoites are released from the thin-walled oocysts soon after the oocysts have been released from the parasitophorous vacuole. Most, but not all, thick-walled oocysts pass through the gut without excysting.

The oocyst stage of the parasite's life cycle is of interest to the water industry. Two forms of oocyst are shed by an infected host (Current 1990). One population is characterized by a thin oocyst wall and is thought to be the primary means for autoinfection. This form of oocyst does not survive for very long outside the infected host. The other form of oocyst is characterized by a robust, thick oocyst wall, and is believed to survive for several weeks in the external environment. This form of the oocyst is the primary means by which this parasite distributes itself throughout a host population. The two autoinfective stages in the life cycle contribute to the severity of the illness and also result in excretion of large number of parasites by infected hosts.

2.1.3 Effects on Human Health

Small numbers of *C. parvum* oocysts can contaminate even treated drinking water, and ingestion of these oocysts can cause diarrheal disease in normal as well as immunocompromised hosts. In a relevant study (DuPont et al. 1995) it was found that an oral dose of 30 *C. parvum* oocysts was found to be sufficient to cause infection in healthy adult humans. The median infective *C. parvum* dose (ID_{50}) was determined to be 132 oocysts. However, for immunocompromised people and children the infective dose is probably much lower.

Unlike immunocompetent adults, people with AIDS are susceptible to a devastating form of cryptosporidiosis manifested by chronic, voluminous diarrhea (Gellin and Soave 1992). *C. parvum* is a recognized cause of diarrhea, particularly among children, in developing countries. Several studies have suggested that cryptosporidiosis is most common in children younger than 1 year (Sallon et al. 1991; Newman et al. 1994).

2.1.4 Waterborne Diseases

Numerous surveys revealed that *C. parvum* is relatively widespread in surface waters (LeChevallier and Norton 1996; LeChevallier et al. 1991; Ongerth and Stibbs 1987; Hayes et al. 1989). In the United States and the United Kingdom, *Cryptosporidium* spp. oocysts have been found in over 80% and 50% of untreated surface waters, and in 26% and 37% of treated drinking waters, respectively (Smith and Rose 1998).

Cryptosporidium parvum has become the most important recognized pathogen in drinking water in the United States (U.S.). Fecal contamination of waterways has led to massive outbreaks. Several well-documented outbreaks of cryptosporidiosis in drinking water in the U.S. affected from 500 to 400, 000 persons (Fayer et al. 2000). Rivers, lakes, springs, and groundwater have all been implicated as sources of this pathogen. About 19 major outbreaks due to cryptosporidiosis have occurred in the U.S., the U.K., and Japan (Smith and Rose 1998). The worst among these was the outbreak in the City of Milwaukee, Wisconsin (U.S.) in 1993 in which as many as 403, 000 persons were affected by this disease.

Cryptosporidiosis outbreaks have occurred in public water systems using conventional treatment processes that were in compliance with federal and local regulations at the time of the outbreak (Solo-Gabriele and Neumeister 1996). The available epidemiological information and the quality of monitoring information are insufficient to estimate the risk of cryptosporidiosis from consumption of potable water (Craun et al. 1998). Analytical risk assessment approaches based on information from the human dose-response studies have often been used to establish the acceptable levels of infectious *C. parvum* oocysts in drinking water and to determine water treatment requirements (Haas et al. 1996; Rose et al. 1991). In North America, in drinking water treatment plants, an estimated 4.5 log-units of oocysts have been estimated to be the average reduction requirements based on an acceptable level of annual infection risk of 1 in 10, 000 persons (LeChevallier et al. 1995).

2.1.5 Detection

The two basic steps involved in oocyst detection are (1) concentration of the oocysts, due to the low numbers of parasites that are typically found in the sample, and (2) identification of oocysts in the concentrated sample (Nieminski et al. 1995). The reference method for concentration of oocysts involves the use of membrane filters (Methods 1622 and 1623: EPA 821-R01-026 and EPA 821-R-99-006). However, the recoveries of the parasites are low and in general range from 9.5% in river water to 59% in tap water (Robertson et al. 1992; Rose 1990).

At present the most accepted technique for detection of parasites in concentrated environmental water samples is the immunomagnetic separation, followed by immunofluorescence (IFA) staining or polymerase chain reactions (PCR) (Di Giovani et al. 1997 a, b). In this technique small magnetic beads with covalently attached anti-*C. parvum* monoclonal antibodies are added to the concentrated water sample. *C. parvum* oocysts present in the sample bind to the antibody and can be removed from the sample by attraction of the bead/*C. parvum* oocyst complex to a magnet. *C. parvum* oocysts are dissociated from the beads through an acid wash (1M HCl). The resulting suspension is then stained with a fluorescently labeled anti-*C. parvum* monoclonal antibody and the preparation examined using epi-fluorescence microscopy (Belosevic et al. 2000). The immunofluorescence method has utility for determining the presence and density of protozoan contamination within a watershed, providing design criteria for calculating the size and complexity of the water treatment process, and can be used to evaluate the effectiveness of cyst reduction through various stages of treatment. Disadvantages of the method include low recovery efficiencies (5 to 25%), long processing times (at best 1 to 2 days, but typically 1 to 2 weeks), the need for a highly trained analyst, high cost (approximately $300 per sample), inability to discriminate viable or virulent strains, and cross-reactivity with several species of *Cryptosporidium* spp. (Fayer 1997).

2.1.6 Viability Determination

In the water industry one of the ongoing challenges is the determination of viability. Dead parasites in finished water are of little concern since they do not have the ability to cause diseases. Ideally, the best test for determining viability is the ability to cause disease in human beings. But, this is unethical and hence animals have been used as surrogates for infection. The technique used for viability determination is called "animal infectivity". However, animal infectivity is tedious, difficult and expensive and is not readily amenable to normal laboratory analysis in the water industry. Further, infections in laboratory animals do not necessarily reflect the infectious process in humans (Belosevic et al. 2000). Although laboratory animals may become infected with *C. parvum* they often do not show overt signs of disease. Nevertheless, animal infectivity is regarded as the "gold standard" for determining the viability of *C. parvum.*

"In-vitro excystation" is another common method used to determine the viability of *C. parvum* oocysts. In-vitro excystation techniques attempt to mimick the conditions of in-vivo digestion. The oocysts excyst in response to proteolytic digestion (usually in the presence of bile salts) resulting in the subsequent release of infectious sporozoites. The number of intact oocysts, empty shells and sporozoites is indicative of the number of viable organisms present in the original suspension (Rennecker et al. 1999). However, there are several assumptions in this method. Firstly, the oocysts that do not excyst are not viable and therefore are incapable of causing infections. But it is possible that those oocysts, which do not excyst in-vitro, may still be infectious. Secondly, it is assumed that the oocysts excysting in-vitro are viable and hence infectious. In the same way it is feasible that the mechanisms mediating oocyst excystation are independent of the viability of the oocyst (Belosevic et al. 2000). It has been reported in the literature that in-vitro excystation often over-estimates viability and infectivity of *C. parvum* oocysts (Black et al. 1996).

Another technique, which is commonly used to measure viability of *C. parvum* oocysts is the in-vitro cell culture assay. In these assays, oocysts are exeysted and the suspension containing infectious sporozoites are inoculated on cultured epithelial cells.

Sporozoites invade the cells and proceed to replicate within the intracellular environment of the cell. Parasites are detected within the cell cultures by a variety of techniques including immunofluorescence (Slifko et al. 1997), polymerase chain reaction (PCR; DiGiovanni et al. 1997a, b), or reverse transcription PCR (Rochelle et al. 1997). The major advantage of the cell culture assays is that the initial establishment of the parasites can be determined. However, the main assumptions regarding in-vitro excystation also apply to the cell culture assays since the latter requires in-vitro excystation as the initial step for initiation of infection.

Other assays for viability determination of *C. parvum* oocysts include immunomagnetic capture PCR (Di Giovani et al. 1997b), dye permeability assays (Campbell et al. 1992; Belosevic et al. 1997a, b) and fluorescence in-situ hybridization techniques (Vesey et al. 1997). Of all the methods, only animal infectivity provides direct information about the ability of a particular parasite to infect the host.

2.1.7 Drinking Water Treatment

Well-designed and properly operated conventional water treatment processes provide physical removal of *C. parvum* to a certain degree. Prevention of waterborne disease in drinking water starts from protection of source waters from contamination. Protected watersheds generally have lower oocyst levels than sites receiving agricultural, sewage, or urban runoff. No studies have quantified the relative contribution of various sources of contamination. Source-water protection is an area where additional research is warranted.

Physical removal of oocysts through coagulation, sedimentation, and filtration is the primary barrier against waterborne cryptosporidiosis (Pett et al. 1994). Effective coagulation of *C. parvum* oocysts has been achieved using alum, ferric chloride and polyaluminium chloride (Nieminski 1994). When coagulation was optimized, an oocyst removal of 2 log-units was demonstrated during both conventional and direct filtration processes, using anthracite coal and sand media (Nieminski et al. 1995; Ongerth and Pecoraro 1995; Patania et al. 1995). It was also found that when coagulation is properly

controlled the total *Cryptosporidium* spp. removal can be improved by an order of magnitude and also the removal is sometimes greater than the total removal of turbidity, particles and spores (Dugan et al. 2001). Non-conventional processes, such as slow sand filtration (Logsdon 1988), dissolved air flotation (Plummer et al. 1995) and diatomaceous earth filtration (Ongerth and Hutton 2001; Schuler and Ghosh 1990; Schuler et al. 1991), have also been shown to be effective for reduction of *C. parvum* oocysts. Microfiltration and ultrafiltration membrane processes can remove all oocysts (Adham et al. 1994). However, application of membranes is currently limited to smaller communities with relatively clean water sources.

In general, treatment of *C. parvum* is more difficult than other pathogens mainly because of its smaller size, lower sedimentation rate and greater resistance to microbial inactivation. In a study (LeChevallier et al. 1996) of 71 surface water treatment plants examined on multiple occasions, oocysts were detected in 39 (54.9%) treatment plant effluents and 15 of the systems were effluent positive in multiple occasions.

Microbial reduction using chemical treatment has always been the major barrier for control of microbial contaminants in water. Chlorine-based chemical treatment of water generally has a low level of effectiveness for oocyst inactivation. As much as 80 mg/L of free chlorine or monochloramine for 90 minutes is required for 1 log-unit oocyst inactivation (Korich et al. 1990). For control of waterborne pathogens a combination of filtration and chemical treatment is necessary. The results of one study (LeChevallier et al. 1996) showed that an average plant would need to provide 2.0 log units of inactivation using chemical treatment along with effective particle removal to meet the 10^{-4} annual risk of *Cryptosporidium* spp. infection goal. This greater resistance of *C. parvum* to chlorination, together with the goal of reducing the formation of health-related chlorination by-products, encouraged the exploration and application of alternative oxidant chemicals like chlorine dioxide and ozone.

2.1.8 Regulatory Requirements

In 1989, the United States Environmental Protection Agency (U.S. EPA) introduced the Surface Water Treatment Rule (SWTR), which established the maximum contaminant level goals (MCLGs) of zero for *Giardia lamblia*, viruses and *Legionella* spp. The SWTR includes treatment technique requirements for filtered and unfiltered systems that are intended to protect against the adverse health effects of exposure to *Giardia lamblia*, viruses, and *Legionella* spp., as well as many other pathogenic organisms. The requirements include the following: (1) maintenance of a disinfectant residual in the distribution system; (2) removal and/or inactivation of 3 log-units of *Giardia* and 4 log-units of viruses; (3) combined filter effluent performance of 5 NTU (Nephlometric Turbidity Unit) as a maximum and 0.5 NTU at 95th percentile monthly for treatment plants using conventional treatment or direct filtration (with separate standards for other filtration technologies); (4) watershed protection and other requirements for unfiltered systems (U.S. EPA 1989).

U.S. EPA promulgated the Total Coliform Rule (TCR) in 1989. The TCR (U.S. EPA 2001) established a MCLG of zero for total and fecal coliform bacteria, and a maximum contaminant level (MCL) based on the percentage of positive samples collected during a compliance period. Under the TCR, no more than 5 percent of distribution system samples collected in any month may contain coliform bacteria (no more than 1 sample per month may be coliform positive in those systems that collect fewer than 40 samples per month). The number of samples to be collected in a month is based on the number of people served by the system.

The Information Collection Rule (ICR), a monitoring and data reporting rule, was promulgated by US EPA in 1996. The ICR (U.S. EPA 2001) required systems to collect source water samples, and in some cases, finished water samples, monthly for 18 months, and test them for *Giardia* spp., *Cryptosporidium* spp., viruses, total coliforms, and either fecal coliforms or *E. Coli*. The ICR also required systems to determine the concentrations of a range of disinfection byproducts in different parts of the treatment plant and

distribution system. The rule also required systems to provide specified operating and engineering data to the U.S. EPA.

The Interim Enhanced Surface Water Treatment Rule (IESWTR) introduced by the EPA in 1998 was to improve the control of microbial pathogens, specifically *C. parvum*, and address risk tradeoffs between pathogens and chemical oxidant byproducts (U.S. EPA 2001). It applies to public water systems serving 10, 000 or more people that use surface water or ground water under the direct influence of surface water (GWUDI). The key features of IESWTR include: a maximum contaminant level goal (MCLG) of zero for *C. parvum*; 2 log-unit of *C. parvum* removal requirements for systems that filter; strengthened combined filter effluent turbidity performance standards of 1 NTU as a maximum and 0.3 NTU at the 95th percentile monthly, based on 4-hour monitoring for treatment plants using conventional treatment or direct filtration and requirements for individual filter turbidity monitoring.

The Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR) is based upon the microbial control provisions established by the IESWTR for large systems through extending similar requirements to small systems (U.S. EPA 2001). The LT1ESWTR applies to public water systems using surface water or GWUDI as sources and which serve fewer than 10, 000 people. Similar to IESWTR, the LT1ESWTR establishes 2 log-unit *C. parvum* removal requirements for systems that filter, and strengthened combined filter effluent turbidity performance standards.

In June 2001, U.S. EPA promulgated the Filter Backwash Recycling Rule (FBRR) to increase protection of finished drinking water supplies from contamination of *C. parvum* and other microbial pathogens (U.S. EPA 2001). The FBRR requirements will reduce the potential risks associated with recycling contaminants removed during the filtration process. The FBRR provisions apply to all systems that recycle the backwash water from the filtration units, regardless of population served.

Current drinking water regulations requiring 2 log-unit removal of *C. parvum* may be adequate for many systems, but U.S. EPA believes that additional protection is needed for systems with greater vulnerability to this pathogen. Such systems include those with high source water *Cryptosporidium* spp. levels and those, which do not provide filtration.

Hence, U.S. EPA has proposed the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) to provide for increased protection against microbial pathogens in public water systems, which use surface water sources. The proposed LT2ESWTR focuses on *Cryptosporidium* spp., which is a protozoan pathogen that is widespread in surface waters. U.S. EPA is particularly concerned about *Cryptosporidium* spp. because it is highly resistant to inactivation by standard treatment practices. In addition to that, the proposed LT2ESWTR is intended to ensure that systems maintain adequate steps to reduce formation of by-products during chemical oxidant treatment of water (U.S. EPA 2001).

2.2 KINETIC MODELING

In the early 20th century, a study (Chick 1908) proposed that the inactivation of microorganisms by chemical agents is a rate-governed process that is analogous to a bi-molecular chemical reaction where one reactant is some vital component in the bacterial protoplasm and the other is the chemical agent. The rate equation was described as:

$$-\frac{dN}{dt} = kN \qquad \text{Equation 2.1}$$

where N is the number of surviving bacteria at a given time and k is the rate constant. Since the rate was also found to be a function of concentration, Watson (1908) proposed the modified rate equation:

$$-\frac{dN}{dt} = kC^{n}N \qquad \text{Equation 2.2}$$

where C is the concentration of the chemical agent and n is known as the coefficient of dilution. n can also be regarded as the number of molecules required to react with one

molecule at a vital component of a microorganism to cause a lethal effect. Integration of Equation 2.2 yields the generalized form of the pseudo first-order Chick-Watson rate law:

$$-\ln\frac{N}{N_0} = kC^n t \qquad \text{Equation 2.3}$$

where N_0 is the initial number of live microorganisms and N is the number of surviving microorganisms after time t. If n is equal to unity, the level of inactivation is proportional to the simple product of the oxidant concentration, C, and the contact time, t.
Expressing equation 2.3 in terms of base 10 logarithmic form:

$$-\log\frac{N}{N_0} = k'C^n t \qquad \text{Equation 2.4}$$

If n is equal to unity, the level of inactivation is proportional to the simple product of the oxidant concentration, C, and the contact time, t.

$$-\log\frac{N}{N_0} = k'Ct \qquad \text{Equation 2.5}$$

Equation 2.5 is simple and extensively used for design and regulatory purposes. U.S. EPA uses the Ct product in Equation 2.5 as the main criteria for design and performance analysis of microorganism reduction processes (Malcolm Pirnie Inc. and HDR Engineering Inc. 1991).

The observed microorganism inactivation often deviates from the Chick-Watson rate law and tend to exhibit non-linear behaviour. *Shoulder* behaviour occurs when the initial rate of inactivation is very low and results in an apparent lag between the addition of the chemical and the onset of measurable inactivation. *Tailing* behaviour is also observed when the rate of inactivation decreases with an increase in exposure. However, it is often not clear whether the differences in reported kinetic behaviour is due to the true

difference in responses due to chemical inactivation of the microorganism, or from the difference in experimental protocols used between the various studies.

Several alternative kinetic models have been proposed. Some of them like the multi-target, series-event and Monod model are based on proposed reaction mechanisms (Gyürék and Finch 1998). Others like the Hom and the rational model are based solely on empirical observations. A few studies have rigorously compared different models, but none of them emerged as a universal solution for all situations. The Chick-Watson model was found to fit as well as the Hom and Monod models for inactivation of coliform bacteria by chlorine when the concentration of chlorine was constant (Haas and Karra 1984a). On the other hand the Selleck model was preferred when chlorine demand and decomposition was significant (Haas and Karra 1984b). The Hom model was found to better represent inactivation of *G. lamblia* cysts by free chlorine than the Chick-Watson model when the two models were compared using maximum likelihood method (Haas and Heller 1990). Zhou and Smith (1994) compared five different models using statistical techniques, representing three basic phenomena for the case of *E. coli* inactivation by ozone in a completely mixed reactor. The models which are more complex than the Chick-Watson model provided only a marginal better fit than the Chick-Watson model with a dilution coefficient of $n = 3.3$. On the other hand, the Hom model was found to be the best among the different models tested, in order to describe the inactivation of heterotrophic plate count bacteria (Gyürék and Finch 1998). In another recent study (Clark et al. 2002), a *Ct* equation based on first order kinetics was developed for the application of ozone for controlling *C. parvum* oocysts in drinking water. The *Ct* equation was developed using standard statistical techniques, and both field and bench scale data. The authors tried to account for the variations in different water types, oocyst strains and nature of bioassay techniques by using a safety factor in their estimation of *Ct* values.

2.3 OZONE IN WATER TREATMENT

The first large-scale installation of ozone generation facilities for drinking water treatment was started in 1893 in the Netherlands. Due to the recognition of chlorination

as a much less expensive alternative, interest in the use of ozone for microorganism reduction declined significantly in North America. However, the new environmental regulations requiring protozoan inactivation and reduction of chlorination by-products have generated a marked increase in the use of ozone for water treatment in the United States.

2.3.1 Reaction and Decomposition of Aqueous Ozone

Ozone chemistry in aqueous solution is complex and the precise nature of the various reactions depends on the type of compounds present in the water. The mechanism of ozone decomposition in water has been a source of significant controversy. However, the models of Staehelin and Hoigné (1982) and by Tomiyasu et al. (1985) are well recognized. Staehlein and Hoigné (1982) concluded that the rate of ozone decomposition in pure water is limited by reaction with hydroxide ions in the initiation step, and hence for a given pH, the decomposition of ozone in pure water should be first order with respect to ozone. Tomiyasu et al. (1985) proposed an expression that included both first and second-order terms to describe the rate of disappearance of ozone in pure waters.

In natural waters, the presence of organic and inorganic matter complicates the determination of ozone decomposition rates. However, it is well established that the rate of decomposition of molecular ozone in natural waters increases with pH and in the presence of hydrogen peroxide, and decreases in the presence of scavenging agents such as carbonate or bicarbonate ions, alkyl groups and tertiary alcohols (Staehlein and Hoigné 1985). Yuteri and Gurol (1988) proposed a first-order rate expression to describe the disappearance of ozone in natural waters. The rate constant of the expression was described as a function of pH, total organic carbon (TOC) and alkalinity of the water.

Hoigné and Bader (1994) proposed a two-step process for describing the ozone decomposition in natural waters. In the first step, ozone is consumed by very rapid reactions within seconds of addition of the ozone to the water. In the second step, a much more gradual decomposition of ozone takes place, which can be approximated by a first-order process. The authors commented that it is generally difficult to predict the rate of

ozone decomposition based solely on the analytical characterization of the water. Oke et al. (1998), however, observed that a first-order decomposition is valid only for clean water. For natural surface waters it provided a poor fit of the ozone decomposition profile. The authors proposed a modified model in which they were unable to correlate the ozone decomposition model parameters with that of the measured water quality parameters.

Ozone reactions in water are influenced by temperature (Morooka et al. 1979; Roth and Sullivan 1983). In general it is difficult to predict the effect of temperatures on the overall ozone decomposition rate in natural waters (Hoigné and Bader 1994). Hence, direct measurement of ozone decomposition in natural waters is preferred rather than prediction.

2.3.2 Mechanisms of Inactivation

In conventional ozonation process, the chemical species primarily responsible for microorganism inactivation is believed to be the molecular ozone. Hydroxyl radicals are not expected to play a significant role as biocidal agents since they are likely to be consumed by reaction with dissolved substrates before they have an opportunity to react with dispersed particles (Hoigné and Bader 1975). Experimental evidence tends to support the molecular ozone hypothesis. It was found that conditions that are favourable for increased hydroxyl radical formation, such as basic pH, UV light and addition of hydrogen peroxide did not enhance inactivation of bacteria or viruses beyond the effect of molecular ozone alone (Farooq et al. 1977; Harakeh and Butler 1985).

In spite of the many experimental studies with ozone, there is little consensus on the mode of action of ozone on microorganisms. Due to chemical selectivity, molecular ozone shows different rates of reaction with different cellular biomolecules. It was found that polysacharrides, phospholipids and amine sugars react slowly with ozone and hence the chemical action of ozone on the cell wall is expected to be weak (Langlais et al. 1991). On the other hand, amino acids and nucleic acids react very rapidly and hence the proteins in the cell membrane and nucleic acids within the cell are potential sites of ozone

attack (Langlais et al. 1991). One study postulated that ozone does not permeate the cells, but rather attacks the bacterial cell wall, alters the cell membrane permeability and finally causes either lysis or leakage of cell components (Scott and Lesher 1963) while others proposed that ozone permeates the bacterial cell membranes and degrades the DNA (Hamelin and Chung 1974; Ishizaki et al. 1987). The primary mode of action of ozone against viruses has been proposed to damage the capsid protein (Kim et al. 1980; Sproul et al. 1982), direct damage of the nucleic acid within the capsid (Roy et al. 1981), or a combination of the both (Shinriki et al. 1988). Hunt and Mariñas (1999) observed that most of the *E. coli* cells exposed to ozone were non-viable even before any structural changes occurred in the cells. This suggested that ozone permeates the cell and the lethal effect is by damage to biochemical molecules or processes within the cell.

Microbial inactivation in natural waters and wastewaters present an additional degree of complexity because ozone will also react with dissolved, colloidal, and particulate matter. These reactions might interfere with some of the reactions responsible for microbial inactivation. Hence, designing disinfection reactors might require the simultaneous consideration of all reactions affecting the concentration of dissolved ozone and ultimately the inactivation process (Hunt and Mariñas 1999).

2.3.3 Inactivation of *C. parvum* on Ozone

Several studies have been done with ozone and *C. parvum* (Peeters et al. 1989; Korich et al. 1990; Ransome et al. 1993; Finch et al. 1993b; Gyürék et al. 1999; Finch and Li 1999; Rennecker et al. 1999; Oppenheimer et al. 2000; Li et al. 2001b). The results of all these studies are complicated by the diversity in the experimental protocols and the variation of the ozonation conditions. In terms of experimental protocol, the differences include the oocyst source host, viability determination method, type of water matrix and the type of reactors used for ozone exposure. Differences may have been due to the specific strain of oocysts used, oocyst purification and storage methods and also the age of the oocysts. Further, the analytical methods used to measure the dissolved ozone and the method of *Ct* calculation may also affect comparisons between studies. All these

resulted in a considerable variation in the *Ct* products required for inactivation of *C. parvum*. This variation creates confusion among water professionals involved in developing engineering design or performance criteria for ozonation systems.

Among all the previous studies with ozone, the most comprehensive were the ones conducted by Finch and coworkers using animal infectivity for oocyst viability determination (Finch et al. 1993b; Finch et al. 1994; Finch and Li 1999; Gyürék et al. 1999; Li et al. 2001b). These researchers used very well-defined protocols, a quantitative mouse infectivity assay to determine the inactivation kinetics of the *C. parvum*-ozone system, and used them to develop engineering design and performance criteria. In their studies the researchers used ultra-pure water to minimize extraneous ozone reactions. They configured a unique batch reactor system that provided continuous monitoring of the dissolved ozone system. Infectivity reduction was measured using a neonatal CD-1 mouse model and was interpreted quantitatively using a logistic oocyst dose-response model (Ernest et al. 1986; Finch et al. 1993a). In the dose response experiments quality control was ensured, by determining the infective properties of each batch of oocysts.

2.4 CHLORINE IN WATER TREATMENT

2.4.1 Chemistry of Chlorine and Chlorine Compounds

Chlorine may be used as a disinfectant in the form of compressed gas under pressure that is dissolved in water at the point of application, solutions of sodium hypochlorite, or solid calcium hypochlorite. The three forms are chemically equivalent because of the rapid equilibrium that exists between dissolved molecular gas and the dissociation products of hypochlorite compounds.

$$Cl_2(g) \Leftrightarrow Cl_2(aq) \qquad \text{Equation 2.6}$$

$$Cl_2(aq) + H_2O \Leftrightarrow H^+ + HOCl + Cl^- \qquad \text{Equation 2.7}$$

$$HOCl \Leftrightarrow OCl^- + H^+$$ Equation 2.8

The term "free available chlorine" is used to refer to the sum of the concentrations of molecular chlorine (Cl_2), hypochlorous acid (HOCl), and hypochlorite ion (OCl^-). As a result of a disproportionation reaction, an aqueous equilibrium exists between the free chlorine species HOCl and OCl^-. The proportion of the species that exist in water, are very much dependent on pH. The majority of the free chlorine is in the HOCl form at pH 6.0, whereas at pH 8.0 the majority exists in the OCl^- form (Haas 1999).

In presence of ammonium ions, free chlorine reacts in a stepwise manner to form chloramines. This process is depicted in the following equations:

$$NH_4^+ + HOCl \Leftrightarrow NH_2Cl + H_2O + H^+$$ Equation 2.9

$$NH_2Cl + HOCl \Leftrightarrow NHCl_2 + H_2O$$ Equation 2.10

$$NH_2Cl + HOCl \Leftrightarrow NCl_3 + H_2O$$ Equation 2.11

The compounds, monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3), each contribute to the total (or combined) chlorine residual in water. The term "total available chlorine", refers to the sum of free chlorine compounds and reactive chloramines. The simultaneous application of chlorine and ammonia or the application of ammonia prior to the application of chlorine, resulting in a stable combined residual, has been a long-standing practice at many utilities (American Water Works Association 1999).

2.4.2 Mechanisms of Inactivation

Several studies have been done on the nature of the inactivation mechanism of chlorine on bacteria, cysts and spores (Fair et al. 1948; Green and Stumpf 1946; Chang

1944). However, the inactivation mechanism of viruses by chlorine and other oxidants has never been resolved. Once taken into the environment of the living organism, chlorine may enter into a number of reactions with critical components causing inactivation (American Water Works Association 1999). In bacteria, respiratory, transport, and nucleic acid activity are all adversely affected (Venkobachar et al. 1975, 1977). In bateriophage f2, the mode of inactivation appears to be disruption of the viral nucleic acid (Dennis et al. 1979). With poliovirus, the protein coat, and not the nucleic acid, appears to be the critical site for inactivation by free chlorine (Fujioka et al. 1985). It is generally agreed that the relative efficiency of various chemical oxidant compounds is a function of the rate of diffusion of the active agent through the cell wall. It is assumed that after penetration of the cell wall is accomplished, the oxidant compound has the ability to attack the enzyme group, whose destruction results in death to the organism (White 1999).

HOCl is the most effective of all the chlorine residual fractions. The germicidal efficiency of HOCl is due to the relative ease with which it can penetrate cell walls. This penetration is comparable to that of water, and can be attributed to both its modest size (low molecular weight) and its electrical neutrality (absence of an electrical charge). The OCl^- ion, on the other hand due to its' –ve charge, is considered to be a relatively poor oxidant (Fair et al. 1948, Chang 1944). There is considerable support for this hypothesis by several investigators (Faust and Aly 1998).

For monochloramine, it was found that for the same conditions of contact time and temperature, and a pH in the range of about 6 to 8, it will take at least 25 times more combined available chlorine than free available chlorine to produce the same germicidal efficiency (Kabler 1953). This difference in potency of monochloramine and HOCl might be explained by the difference in their oxidation potentials, assuming that the action of chloramines is of an electrochemical nature rather than one of diffusion as in the case of HOCl (White 1999). In a different study (Jacangelo et al. 1991) the mode of action of monochloramine on *E. coli* B was found to be inhibition of typical protein-associated biological activities such as bacterial transport, respiration, and, substrate dehydrogenation.

2.4.3 Inactivation of *C. parvum* by Chlorine

Several studies have confirmed that free chlorine alone is not effective against *Cryptosporidium parvum* at doses and contact times that are commonly used in water treatment (Korich et al. 1990; Ransome et al. 1993; Gyürék et al. 1997). The data in these studies indicated that chlorine can achieve some sort of inactivation of *Cryptosporidium* spp., but at very high concentrations and contact time.

Korich et al. (1990) used oocysts in oxidant demand free phosphate buffer for chlorine treatment. Viability was determined using Balb/c mice, in-vitro excystation and vital dyes. The authors reported 2 log-units of inactivation of oocysts exposed to 80 mg/L of chlorine for 90 minutes. Ransome et al. (1993) tested inactivation of *C. parvum* in groundwater buffered at a pH of 7 at 10°C. Viability of the oocysts was determined using excystation. Less than one log-unit of reduction in viability was achieved at an approximate dose of 1000 mg/L of free chlorine for 24 hours. A comprehensive investigation was done by Gyürék et al. (1997) using *C. parvum* oocysts in demand-free phosphate buffer at a pH of 6 and 8 maintained at 25°C. Viability was measured using animal infectivity. The authors reported 0.5 to 1 log-unit inactivation of the oocysts at pH 6 for *Ct* values ranging from 60 to 1,032 mg.min/L. Less than 0.5 log-unit of inactivation was observed for *Ct* values ranging from 60 to 240 mg·min/L.

In spite of the inability of chlorine to effectively inactivate *C. parvum,* the number of utilities using chlorine seems to remain unchanged from 1978 to 1998 in about 165 large and medium sized utilities in the United States (AWWA Water Quality Division Disinfection Systems Com., 2000).

Much lower credit is given for microorganism reduction per unit of chloramines residual per unit contact time compared to chlorine, chlorine dioxide and ozone. However, chloramines have been increasingly recognized as an effective means to control by-products of chemical treatment and biofilm growth in distribution systems despite their poor instantaneous biocidal efficacy.

Korich et al. (1990) reported a one log-unit of *C. parvum* inactivation after exposure of 80 mg/L of monochloramine for 90 minutes in demand-free phosphate buffer

at a pH of 7 and 25°C. Ransome et al. (1993) found a reduction in excystation of approximately 73 % after the treatment of oocysts at 3 mg/L of monochloramine for 24 hours. Gyürék et al. (1997) studied the effect of monochloramine on oocysts at 22°C in phosphate-buffered water at pH 8. The authors reported *Ct* values ranging from 3, 300 to 7, 000 mg·min/L to achieve 1-log unit inactivation of oocysts.

2.5 SEQUENTIAL TREATMENT FOR MICROORGANISM REDUCTION

A number of studies have been conducted on the use of multiple oxidants, for inactivation of microorganisms in drinking water applications. In earlier studies, mixtures of oxidants were investigated typically in simultaneous application to an aqueous solution. Kouame and Haas (1991) demonstrated a synergistic effect on inactivation of *E. coli* when free chlorine and monochloramine were both present in a continuous stirred tank reactor system at pH 8 and 20°C. These authors noted that the synergistic effect increased with contact time. Katz et al. (1994) studied the effect of chlorine dioxide and chlorine combinations on the inactivation of indicator organisms contained in activated sludge effluent. These authors reported improved inactivation of total coliforms, fecal coliforms, fecal streptococci, and coliphages following combined application of 5 mg/L each of chlorine dioxide and chlorine, but they did not report the inactivation from separate application of the disinfectants. It is therefore, difficult to assess a synergistic effect from their results. Kott et al. (1980) studied the effects of ozone and chlorine applied individually, sequentially and combined on the inactivation of *Salmonella typhimurium*, poliovirus type 1 and T_2 and T_3 coliphages in secondary wastewater and artificially polluted tap water. These authors reported that simultaneous application of ozone and chlorine was superior to sequential application.

A proprietary device that produces an ill-defined mixture of chemical oxidants purported to include free chlorine, ozone and chlorine dioxide, has been reported to cause substantial inactivation of indicator bacteria, anaerobic spores and encysted parasites (Sobsey et al. 1998; Venczel et al. 1997). Recent chemical analysis confirmed high concentrations of free chlorine but could not detect ozone or chlorine dioxide in the

mixed oxidant solution (Bubnis et al. 1998). In another study, where the conditions reported by Venczel et al. (1997) were repeated, the inactivation of *Cryptosporidium* spp. was no different than that expected from free chlorine alone. While the two studies appeared to use the same protocols for the experiments, Venczel et al. (1997) used a tissue culture assay to determine the viability of the oocysts.

Finch et al. (1995) reported superior inactivation of *Cryptosporidium* spp. oocysts when using free chlorine followed by monochloramine in deionized water at room temperature when compared to either disinfectant alone. This led to a series of preliminary experiments studying the phenomenon (Finch et al. 1997; Gyürék et al. 1997). A more complete investigation of chemical treatment combinations reported that synergistic effects on *Cryptosporidium* spp. may be hindered at cold temperatures (Finch et al. 1997). However, once a threshold *Ct* of the primary oxidant was applied, there was a progressive increase in inactivation of *Crytposporidium* spp. with increasing *Ct* of the secondary disinfectant. Ozone-based processes were found to be the best followed by chlorine dioxide processes.

Driedger et al. (1999) studied ozone followed by monochloramine sequential inactivation of *C. parvum* oocysts using in-vitro excystation as a viability assay. The authors reported a five-fold increase in the inactivation rate for monochloramine after 0.26 log-units of ozone primary treatment (ozone Ct = 1.4 mg.min/L) versus no primary treatment. Rennecker et al. (2000a and 2001) and Driedger et al. (2001) used an *in vitro* excystation assay to study sequential inactivation of *C. parvum* oocysts suspended in de-ionized water in batch reactors. They observed that semi-log curves of inactivation ratio versus free or combined chlorine concentration-time (Ct) product were characterized by an initial lag phase, in which little inactivation occurred, followed by a region at higher Ct in which the inactivation rate appeared to follow a first-order kinetic rate law. In addition to direct oocyst inactivation, pre-treatment with ozone reduced the duration of the lag phase and increased the post-lag first-order rate of inactivation during subsequent exposure to free or combined chlorine. Gross oocyst inactivation was greater than that predicted based on simple addition of the inactivation expected for each chemical acting alone and a synergistic effect was claimed. Recently, the same research group (Corona-

Vasquez et al. 2002) found that the kinetics of primary inactivation with ozone and free chlorine has a relatively strong temperature dependence (increases with decreasing temperature), and vary both with oocyst lot and oocyst age.

Finch et al. (2000) and Li et al. (2001b) used mouse infectivity assays to measure the viability of *C. parvum* oocysts suspended in phosphate buffered de-ionized water and exposed to various combinations of chemical oxidants. Although they did not observe the initial lag phase reported by the researchers using the in vitro excystation assay, they concluded that pre-treatment with ozone increased the first-order rate of inactivation during subsequent exposure to free and combined chlorine and thereby generated a measurable synergistic effect. Finch et al. (2000) found that ozone followed by free chlorine at pH 6 or ozone followed by monochloramine at pH 8 generated a measurable synergistic effect on the inactivation of *C. parvum* oocysts. Gross inactivation with sequential treatment increased linearly with the free chlorine *Ct* product. A decrease in temperature reduced the magnitude of the synergistic effect. Li et al. (2001b) conducted a series of sequential treatment experiments with different combinations of disinfectants to determine the synergistic effect in buffered de-ionized water. A significant synergistic effect was observed for ozone-based sequential inactivation, and some extra benefit was also found for chlorine dioxide-based sequential inactivation at high *Ct* of the secondary treatment. The factors that were found to significantly affect sequential inactivation included the level of primary inactivation, the *Ct* product of the secondary treatment and the temperature of the water. The gross inactivation after primary treatment was observed to increase linearly with the *Ct* product of the secondary treatment. Both the gross kill and the synergistic effect were favored at higher water temperatures. For 1.6 log-units of ozone primary kill, the efficacy of free chlorine or monochloramine secondary treatment on a total available chlorine basis was comparable.

Very few studies on synergistic effect of disinfectants on *C. parvum* oocysts suspended in natural waters have been reported. A number of natural water experiments at a variety of temperature and pH combinations using ozone alone and chlorine dioxide alone and in combination with free chlorine and monochloramine have been performed at the laboratories of the Environmental Engineering and Science Program and the

Department of Biological Science of the University of Alberta. Many of these proprietary studies are not in the public domain. A general observation was that synergistic effects were apparent, but not at the levels expected based on studies conducted in buffered deionized water. Temperature and water quality were speculated as factors affecting the outcome.

Oppenheimer et al. (2000) conducted a limited number of sequential disinfection experiments with *C. parvum* oocysts suspended in different natural waters and using a mouse infectivity assay to determine oocyst viability. In their studies, they found some evidence of a synergistic effect when chlorine or chloramines were applied following ozone primary treatment. However, the synergistic effect was inconsistent in the various waters tested. They cited the limited precision of the animal infectivity assay and a poor understanding of the factors responsible for the synergistic effect in the natural water environment. The Oppenheimer et al. (2000) study is the only reported study of sequential treatment of *C. parvum* in natural waters.

2.6 HYPOTHESIS OF SYNERGY IN NATURAL WATERS

A general hypothesis for the mechanism of synergy is that the strong oxidants in the primary treatment increase the permeability of the oocyst wall by physically damaging or altering its' surface properties. Without pre-treatment, diffusion of both free chlorine and monochloramine through the oocyst wall is the rate-limiting step in the diffusion-reaction process. Since ozone is a very strong oxidant, and it can oxidize many organic materials including lipid and proteins, it was postulated earlier (Li et al. 2001b) that the ozone pre-treatment increased oocyst wall permeability by weakening the oocyst wall. For natural waters, a similar mechanism will probably be true. However, the microorganisms may acquire physical protection in water as a result of their being adsorbed to the enormous surfaces provided by clays, silt, and organic matter. Such particles, with the adsorbed microorganisms, may aggregate to form clumps. Organisms themselves may also aggregate or clump together so that the organisms that are on the interior of the clump are shielded from the disinfectant and are not inactivated.

Organisms may also be physically embedded within particles of fecal material, or within larger organisms (National Academy of Sciences, 1980). Microbial inactivation in natural waters using chemical oxidants, also presents an additional degree of complexity. The oxidants react with dissolved, colloidal, and particulate matter, and these reactions might interfere with some of the reactions responsible for microbial inactivation.

CHAPTER 3: MATERIALS AND METHODS

3.1 PARASITOLOGY METHODS

3.1.1 Production of *C.parvum* Oocysts

C. parvum oocysts used in this study were originally obtained from Dr. Harley Moon (National Animal Disease Control Center, Ames, Iowa) and are known as the Iowa strain. Previously established methods for *C. parvum* oocyst production and purification from Holstein calves were used (Finch et al. 1994; Finch et al. 1995; Finch et al. 1997). Calves aged 2 to 4 days were infected with *C. parvum* oocysts and maintained on a diet of electrolyte solution. Feces collected from the calves were first passed through descending series of sieves (400μm to 75 μm). Oocysts were purified from the sieved feces by cesium chloride gradient centrifugation (Kilani and Sekla 1987). The calf diet of electrolyte solution reduced the lipid content of the feces and eliminated the need for a sucrose centrifugation pre-purification step. Stock suspensions of purified oocysts were stored at 4°C in deionized water with antibiotics (100 μg/mL streptomycin, 100 μg/mL gentamicin, 100 U/mL penicillin) and 0.01% Tween.

3.1.2 Oocyst Sample Concentration

Samples of oocysts from experimental trials were centrifuged at 27, 000 × *g* for 10 min to concentrate the oocysts for inoculation into neonatal CD-1 mice. The supernatant was aspirated and the cell pellet re-suspended in deionized water. Oocysts were counted in quadruplicate using a hemacytometer and appropriate dilutions prepared in deionized water for mouse infection. For each experimental sample, 2 to 3 dilutions of oocysts were prepared and cohorts of 5 mice were inoculated with each dilution. A typical dilution set would result in inoculations of 1, 000; 10, 000; and 100, 000 oocysts per mouse. Control samples of the experimental parasites were subjected to all the same

processing and handling steps applied to the treatment samples, with the exception of exposure to the treatment chemicals. Controls were used on each experimental day.

3.1.3 Infectivity in Neonatal CD-1 Mice

A neonatal mouse model was used to evaluate infectivity of *C. parvum* (Ernest et al. 1986; Finch et al. 1993). Breeding pairs of outbred CD-1 mice were obtained from the Charles River Breeding Laboratories (St. Constant, Quebec, Canada). The neonates were housed in litters with a lactating dam. The animals were given food and water; and were housed in cages with covers fitted with a 0.22 µm filter in a specific pathogen-free (P-2 level) animal facility. Mice were inoculated intragastrically five days after birth with a known number of oocysts suspended in 50 µL of deionized water. Intragastric inoculation was preformed using a ball-point neonate feeding needle (24 gauge syringe, Popper and Sons Inc., New York, USA) attached to a tuberculin syringe.

The infectivity of the oocysts was determined 7 days after infection. The mice were killed by cervical dislocation and the large intestine (rectum to 30 mm anterior to the caecum) was removed and placed in 10 mL of Milli-Q® water. The intestine was homogenized for 45 to 60 s in a Sorvall Omni-Mixer and the homogenate placed in a 15 mL polypropylene test tube. The suspension was centrifuged at 2000 × *g* for 15 min. The supernatant was then removed and the pellet was re-suspended in 10 mL of deionized water containing 0.01% Tween 20 and centrifuged at 2000 × *g* for 15 minutes. After centrifugation, the supernatant was discarded and 20 µL of the viscous pellet was removed and placed into a 6 mL polystyrene flow cytometer test tube fitted with a 35 µm sieve (Becton Dickinson). The intestinal homogenate was forced through the sieve by adding 400 µL of 1% bovine serum albumen (BSA) in phosphate buffered saline (PBS). Samples were allowed to incubate for 15 minutes at room temperature, in order to block non-specific absorption of the monoclonal antibody. One hundred µL of a 1:400 dilution of fluorescein labeled anti-*C. parvum* oocyst monoclonal antibody (ImmuCell), diluted in 1% BSA, was subsequently added to each sample and incubated at 37°C for 30 minutes. The resulting suspension was examined for the presence of parasites using flow

cytometry (Neumann et al. 2000). Settings for the flow cytometer (FACSCalibur, Becton-Dickinson, San Jose, CA) were as follows: forward side scatter – photodiode voltage equivalent to E00, AmpGain 4.00; side light scatter – photomultiplier voltage set to 402, AmpGain 4.00; FL1 - photomultiplier voltage set to 470. All flow cytometric analysis was done at a high flow rate using PBS as the sheath fluid. Fifty thousand events were collected for each intestinal homogenate sample. The maximum number of events failing into the specified regions (i.e. size, internal complexity, and fluorescence) from uninfected mouse intestinal homogenates was used as the baseline criterion for determining whether a mouse was infected with *C. parvum*. This upper limit corresponded to a value of 1.25% of the gated events. Mouse homogenates having gated events greater than this value were scored as infected, and those less than or equal to this value were scored as non-infected. At regular intervals (4 months), flow cytometric results were confirmed using conventional microscopy methods (Neumann et al. 2000).

3.2 CHEMICALS AND APPARATUS

3.2.1 Chlorine

Free chlorine stock solution was prepared daily by adding an appropriate aliquot of purified sodium hypochlorite solution (6% available chlorine, BDH Inc., Poole, England) into oxidant demand-free de-ionized water to give a concentration of about 300 mg/L. Chlorine concentration was determined by the DPD procedure (Eaton et al. 1995); and occasionally in some trials were checked by the forward amperometric titration method (Eaton et al. 1995) using a Cl Titrimeter Fisher Scientific model 397 and phenylarsine oxide. The stock chlorine solution was stored at 4°C in dark refrigerated conditions during the day of the experiment.

3.2.2 Monochloramine

Ammonium chloride stock solution (1000 mg/L, AnalaR grade, BDH Inc., Poole, England) was used to prepare monochloramine solutions for use in experiments. A daily

working monochloramine solution was prepared by mixing appropriate volumes of sodium hypochlorite and ammonium chloride stock solutions in pH 8.0 oxidant demand-free buffer solution to yield a 150 ± 10 mg/L combined chlorine solution with a chlorine:nitrogen weight ratio of 3:1. After initial mixing, the solution was stirred for 30 minutes. The concentration of free chlorine and combined chlorine were then measured using the DPD procedures for free and total chlorine; and occasionally were checked by the forward amperometric titration method (Eaton et al. 1995) using a Cl Titrimeter (Fisher Scientific model 397, Edmonton, AB) and phenylarsine oxide. Chlorine colorimetric DPD measurements were found to be approximately 0 to 6 % higher relative to amperometry for both free chlorine and total chlorine. This discrepancy in chlorine measurements between DPD and amperometry has been reported elsewhere (Gordon et al. 1992). The DPD measurements were eventually used for the experiments, as it was very convenient and easy to measure. For preformed monochloramine stock solutions, the fraction of total chlorine measured as free chlorine, following 30 minute of mixing, using the colorimetric DPD and amperometry was 0 to 2 % and 0 to 3 % respectively. The free available chlorine concentration was subtracted from the total available chlorine concentration to determine the monochloramine concentration in the stock.

3.2.3 Ozone

Ozone gas was generated from extra dry oxygen feed gas using a water-cooled corona discharge generator (Model T-816, Welsbach Ozone Systems Corporation, Sunnyvale, CA). Concentrated ozone stock solution was prepared by bubbling oxygen carrier gas containing approximately 4 percent ozone (v/v) through 400 mL of refrigerated (4°C) deionized water for a minimum of 20 min. Ozone concentration in the stock solution was approximately 40 mg/L. The ozone solution was used within 3 min of removing it from the gas stream. Ozone residual concentrations in the stock were determined by direct ultraviolet absorbance measured at 260 nm on a diode-array spectrophotometer (HP 3452A Hewlett Packard Co., Wilmington, DE). A molar absorption coefficient of 3, 300 $M^{-1} cm^{-1}$ was used (Hart et al. 1983).

3.2.4 Oxidant Demand-Free Glassware

All glassware used in experiments was initially cleaned using a detergent specifically designed (Sparkleen 2, Fisher Scientific Co., Pittsburgh, PA) for laboratory glassware. After the initial cleaning all glassware was rinsed three times with deionized water including an acetic acid rinse. The disinfection reactor, stir bar, pipette tips and other glassware that made contact with the test solution were made ozone demand-free before use. It was found earlier (Li et al. 2001b) that ozone demand-free water and utensils were also chlorine demand-free. Therefore, laboratory procedures were streamlined to follow the ozone demand-free protocols and calling the prepared water and utensils "oxidant demand-free" (ODF). Openings in the glassware were covered with fresh aluminum foil to prevent dust from entering the glassware.

3.2.5 Reactor Vessels

The reactor vessels used for all experiments were 250 or 500 mL Erlenmeyer flasks that were made oxidant demand-free. Parasite suspensions in the flask were stirred using a Teflon-coated magnetic stir bar. The agitation speed was sufficient to ensure rapid and complete mixing of the added chemicals but without creating a significant vortex.

When working with ozone, free chlorine and monochloramine, reactor vessels were covered with aluminum foil to minimize volatilization and photodecomposition of disinfectants. A diode-array spectrophotometer (Hewlett-Packard Model 8452A, Palo Alto, CA) with a 10 mm light path and 35 μL flow through cell was operated in a closed loop to continuously monitor the ozone concentration in the flask as described elsewhere (Finch et al. 1994; Li et al. 2001b). A molar absorption coefficient of 3, 300 M^{-1} cm^{-1} was used (Hart et al. 1983).

3.2.6 Temperature Control

Temperature in the reactor was controlled by a water bath with a microprocessor control (Model 3545, Lab-Line Instruments Inc., Chicago, IL). When low temperature was needed for the experiment, the water bath was maintained at a constant low temperature by using submerged cooling coils connected to a separate refrigerator unit. During experiments, the reactors were submerged in the water bath. Stirring for individual reactors was provided by submersible magnetic stirrers (Model 230, VWR Canlab, Mississauga, Ont).

3.2.7 pH Adjustment

Prior to each experimental trial, the pH of the water sample to be used was measured using an Accumet Model 25 pH/Ion Meter (Fischer Scientific). For those trials conducted at lower pH, 1 M sulfuric acid was added to the water sample with constant stirring until the pH of the water stabilized at the desired level. The pH was left to stabilize at the target overnight and was adjusted if necessary.

3.3 SCOPE AND STUDY APPROACH

The data in this study were collected in controlled bench-scale experiments using prepared oocysts suspended in different natural surface waters. The natural waters chosen for experiments varied significantly in the measured water quality parameters. Animal infectivity using an established neonatal CD-1 mouse model was used to measure oocyst viability before and after treatment. The magnitude of the synergistic effect was interpreted according to the *Ct* analysis depicted in Figure 3.1. This hypothetical figure shows the measured infectivity reduction as a function of the secondary *Ct* product. The level of inactivationresulting from exposure to the secondary chemical, either with or without ozone primary treatment, was considered to be proportional to the *Ct* value. The measurements shown at a *Ct* of zero for the sequential treatment represent the infectivity

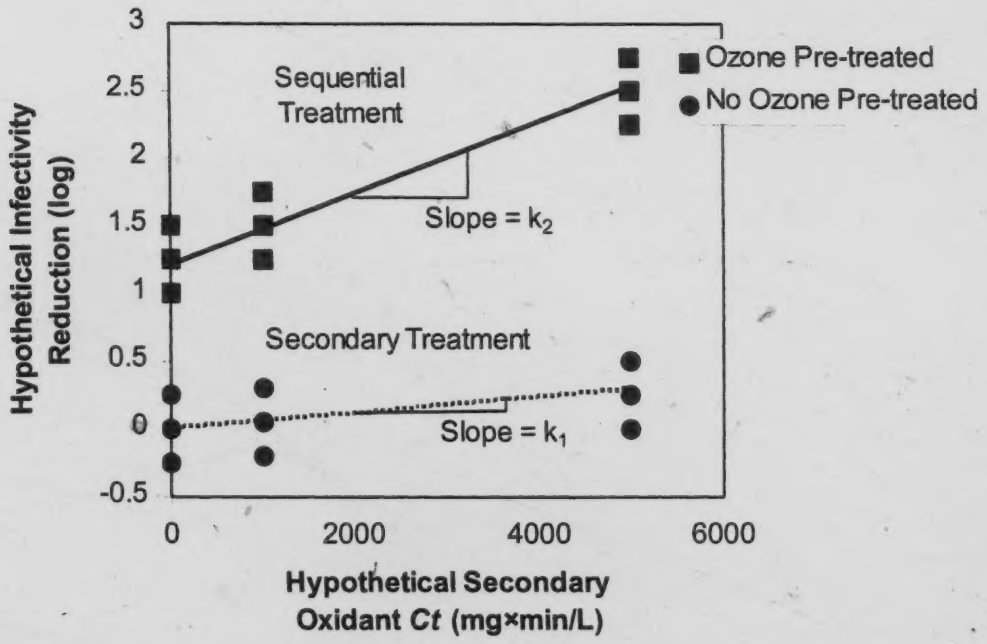

Figure 3.1 Illustration of the *Ct* approach used to measure a synergistic effect. The hypothetical data set shown in this chart would have been obtained for a single natural water, pH, temperature and primary ozone treatment level. A synergistic effect is measured when $k_2 > k_1$.

reduction after application of the primary oxidant (ozone), but before addition of the secondary oxidant.

The *Ct* experimental approach was adopted for measurement of a synergistic effect in this study versus single point measurements. With replicated experimental trials, as illustrated in Figure 3.1, confidence intervals on the values of k_1 and k_2 were determined and inferences about the statistical significance of the synergistic effect were made. Measurement of infectivity loss at both high and low *Ct* values provided a check on the validity of the linear assumption.

3.4 PROTOCOL FOR EXPERIMENTAL TRIALS

Approximately 130 to 200 mL volume of water sample and a preparation of oocysts (25×10^6 or 50×10^6) were added to each reactor. The reactor was placed in the water bath, and stirred. The suspension was left for 1/2 hour to equilibrate to the target temperature. The protocols for sequential (ozone followed by free chlorine or monochloramine) treatment, secondary disinfectant treatment (free chlorine or monochloramine alone) and control treatment (no chemicals added) were as follows:

Sequential Treatment, Reactor 1: At the start of the primary treatment, an aliquot of ozone stock solution was transferred to the stirred suspension in the reactor using a pipett (Oxford Macroset Labware, St. Louis, MO) fitted with an ODF tip. The volume of ozone stock solution added was based on the target initial ozone concentration for the particular experiment. During the course of the ozone contact period, four 10 mL samples of water were extracted from the flask using a similar pipette fitted with an ODF tip. The samples were immediately analyzed for dissolved ozone using the indigo-trisulphonate method with a molar absorbance coefficient of 20 000 $M^{-1}cm^{-1}$ (Eaton et al. 1995). For natural waters, the indigo-trisulphonate method was preferred over direct UV absorbance at 260 nm for measurement of dissolved ozone concentration. Although both methods were used, the latter method was seriously limited by interference from absorbing substances present in some natural waters. The measured ozone concentration-time profile in the reactor was fitted to a first-order decay equation of the form $C = C_0\exp(-k_d t)$. For each experimental trial, the initial ozone concentration, C_0, and the first-order decay rate constant, k_d, were estimated using least-squares regression. After the desired contact time, sodium formate (200 µL of 1M) was added to quench the remaining ozone. At this point a 40 mL sample was extracted from the flask for *C. parvum* oocyst infectivity analysis to determine the level of inactivation after ozone treatment.

An aliquot of stock free chlorine or monochloramine solution was then added to obtain the target initial free or combined chlorine concentration. After 3h, a 40 mL sample was collected for infectivity analysis. After an additional 2h or 13 h, sodium

sulphite was added to neutralize any remaining free chlorine or monochloramine and the remaining flask contents were collected and concentrated for infectivity analysis. Free chlorine or monochloramine concentrations were determined at the beginning of the exposure period (immediately after chlorine addition), after 3 hours and at the end of the exposure period (prior to sulphite addition). The concentrations were determined at the beginning (C_0) and at the end (C_f) of the contact time using the DPD (*N,N*-diethyl-*p*-phenylenediamine) free chlorine or total chlorine methods (Eaton et al. 1995). DPD reagents were supplied by Hach Co. (Loveland, MI). Using these measured concentrations, the geometric average free chlorine or total chlorine concentrations were determined as follows:

$$C_{avg} = \sqrt{(C_0 \times C_f)} \qquad \text{Equation 3.1}$$

The geometric mean was chosen for determining the chlorine concentrations because this is the most appropriate representation of integrated chlorine exposure for 1st order decay. The first-order decay of chlorine was verified in preliminary experiments on the natural waters. Use of an arithmetic average would have introduced a bias, although a relatively small one, into the determination of $C_{avg}t$.

Secondary Treatment Alone, Reactor 2: Exposure of the oocysts to the secondary chemical alone was carried out in a manner identical to the sequential treatment with the exception that ozone addition, ozone concentration measurement and the post-ozone oocyst sample collection steps were eliminated.

Control, Reactor 3: This reactor flask was maintained under constant stirring at the same temperature as the other two flasks and for the same total sequential inactivation contact time (ozone + chlorine or monochloramine) as reactor 1. No ozone, chlorine and monochloramine were added. Aliquots of sodium formate and sodium sulfite were added at the same times as in reactor 1. Reactor contents were collected at the end of the

exposure period for oocyst infectivity analysis. The control trials were done only to determine if any significant inactivation of the oocysts occurred due to natural water alone or due to oocyst storage, handling and processing steps.

3.5 KINETIC MODELING

3.5.1 Interpretation of Infectivity Data with the Logistic Dose Response Model

The reduction in oocyst infectivity was interpreted as the infectivity reduction i.e. $-\log(N/N_0)$, where N is the number of infective oocysts in the suspension after exposure to the oxidant chemicals and N_0 is the number prior to exposure. For each trial, the inactivation ratio (in log-units) was estimated from

$$-\log\left(\frac{N}{N_0}\right) = \log\left(\frac{d}{d_0}\right) \qquad \text{Equation 3.2}$$

where d is the estimated number of infectious oocysts in the inoculum of each mouse trial and d_0 is the total number of oocysts in the same inoculum as determined by hemacytometer count. The proportion of mice infected 7 days post-inoculation, P, was determined for each cohort using previously described methods. The estimated infectious dose, d, was then estimated using a logistic dose response model for *C. parvum* oocyst infectivity in the neonatal CD-1 mice. The form of the model is (Neter et al. 1989):

$$\pi' = \ln\left[\frac{P}{(1-P)}\right] = \beta_0 + \beta_1 \log d \qquad \text{Equation 3.3}$$

In Equation 3.3, P is the proportion of animals in a cohort that become infected subsequent to ingesting a specified live inoculum, d, of oocysts. The parameters of the logistic model, β_o and β_1, were determined for each batch of oocysts used in the experimental trials in oocyst dose response experiments. For dose reponse, cohorts of 10 neonatal CD-1 mice were inoculated with levels of inoculums ranging from 25 to 200

oocysts (1 cohort per inoculum level) per mouse. The number of animals positive for infection at each dose level was determined using previously described methods. Parameters of the logistic model were estimated from the results of the dose response experiments by maximizing the natural logarithim of the likelihood function, ln *L*, for binary data, given by Brand et al. 1973:

$$\ln L = \sum_{i=1}^{\alpha} Y_i(\beta_0 + \beta_1 X_i) - \sum_{i=1}^{\alpha} \ln[1 + \exp(\beta_0 + \beta_1 X_i)] \qquad \text{Equation 3.4}$$

In Equation 3.4, the subscript i = 1, 2, ... α represented each individual mouse used in the dose response experiment, X_i was the inoculum size for each mouse, and Y_i was the binary score (0 = not infected, 1 = positive) of each mouse after 7 days. The likelihood function was maximized and the model parameters estimated using the Solver function in Microsoft Excel 98®.

Dose response experiments were conducted for each batch of oocysts used in these experiments and during each experimental week. For a given batch of oocysts, the results of the weekly dose response experiments were pooled and analyzed by the methods described above. The *C. parvum* oocysts used in this research came from 8 batches. The ID_{50} (the infectious dose which caused the infection of 50% of the CD-1 mice cohort) and their logistic dose-response model parameters are summarized in Table 3.1. The 90% confidence limits of the model parameters, β_0 and β_1, are also provided in the Table 3.1.

The ID_{50} in neonatal CD-1 mice ranged from 54 to 205 oocysts per animal. In an earlier study (Gyürék et al. 1999), an ID_{50} of 60 to 347 oocysts per animal for *C. parvum* infection was reported. The variations in ID_{50} among the different batches of oocysts confirmed earlier findings that the dose-response is batch specific. Individual dose-response models for each batch of oocysts are, therefore, necessary to ensure a high quality infectivity analysis.

Table 3.1: Logistic dose-response models for neonatal CD-1 mice exposed to different batches of *C. parvum* oocysts

Batch No.	Number of mice used for the model	Number of cohorts	$\hat{\beta}_0$ (± 90 % CI[a])	$\hat{\beta}_1$ (± 90 % CI[a])	ID_{50} (± 90 % CI[a]) (oocysts per mouse)
35	269	31	-9.89 (-10.2, -9.6)	5.17 (5.0, 5.3)	82 (65, 110)
36	159	16	-5.63 (-5.9, -5.3)	3.21 (3.0, 3.4)	57 (36, 93)
37	120	12	-8.48 (-8.8, -8.1)	4.77 (4.6, 5.0)	60 (42, 82)
38	240	24	-4.80 (-5.0, -4.5)	2.78 (2.7, 2.9)	54 (36, 71)
39	240	28	-4.33 (-4.5, -3.8)	2.06 (1.9, 2.3)	126 (45, 234)
40	199	20	-5.38 (-5.6, -5.1)	2.68 (2.6, 2.8)	102 (66, 143)
41	320	32	-6.64 (-6.9, -6.4)	3.26 (3.1, 3.3)	108 (87, 168)
60	80	8	-6.40 (-7.8, -6.9)	2.77 (3.0, 3.4)	205 (108, 376)

[a]CI=Confidence Interval

3.5.2 Chick-Watson Model and the I.g.H Model

A generalized microorganism inactivation rate law that includes first-order disappearance of the disinfectant chemical can be written as (Gyürék and Finch, 1998):

$$\frac{dN}{dt} = -k'' m N^x C_0^n e^{-k_d n t} t^{m-1} \qquad \text{Equation 3.5}$$

where dN/dt = rate of microorganism inactivation; k''= inactivation rate constant for the particular microorganism; N = number of infective microorganisms at the contact time t (min); C_0 = the initial concentration of the chemical (mg/L); k_d = the rate constant of the first-order disinfectant decay (min^{-1}); and m, n, x = empirical constants. Assuming $x = m = 1$, and integrating the equation yields the following equation for inactivation under conditions of first-order disappearance of the chemical:

$$\log\frac{N}{N_0} = -\frac{k^{''}}{k_d n}(C_0^n - C_f^n) \qquad \text{Equation 3.6}$$

where C_f = chemical concentration at the end of the contact time (mg/L) and is calculated as $C_f = C_0\exp(-k_d t)$. The model can be simplified by assuming n = 1 and a constant chemical concentration. This reduces to the well-known Chick-Watson form where inactivation is expressed in terms of an average Ct model product:

$$\log\frac{N}{N_0} = -kC_{avg}t \qquad \text{Equation 3.7}$$

where C_{avg} is some average measure of the disinfectant concentration. In the case where n and m are not unity, but x is, integration of Equation 3.5 gives the Incomplete Gamma Hom (I.g.H) model, which can be used to describe the shoulder effect and tail-off effect of the inactivation curves (Haas and Joffe, 1994):

$$\log\frac{N}{N_0} = -\frac{mkC_0^n}{(nk_d)^m}.\gamma(m, nk_d t),\ m > 0,\ nk_d t \geq 0 \qquad \text{Equation 3.8}$$

The incomplete gamma function, $\gamma(m,nk_d t)$, can be conveniently solved in Microsoft Excel 98 using the following combination of statistical functions: GAMMADIST ($nk_d t$, m, 1, true) × EXP(GAMMALN(m)).

3.6 STATISTICS

3.6.1 Multiple Linear Regression Analysis

To determine which experimental factors had a significant impact on the synergistic effect, a model of the following form was regressed to the outcomes of the experiments:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 \qquad \text{Equation 3.9}$$

where a_0, a_1, a_2, a_3 and a_4 represent the model parameters; X_1, X_2, X_3 and X_4 represent the independent variables and Y represents the dependent variable in the equation. Multiple regression analysis was done using the regression tool in Microsoft Excel 98 (Draper and Smith, 1966). Model terms were rejected or retained based on the statistical significance of the coefficients a_0, a_1 ...etc. at the 90% confidence level (backward elimination technique). For a model term to be considered significant at the 90% level, the computed p-value for the associated parameter, a_1, was less than 0.1. (Note: the p-value is the probability of making a Type I error in a hypothesis test in which the null hypothesis is $a_i = 0$).

3.6.2 Confidence Interval of the Difference Between Two Slopes

In order to compare the slopes (k_2 and k_1) of two linear lines, the following test statistic was used (Zar 1984):

$$t = \frac{k_2 - k_1}{s_{b_2 - b_1}} \qquad \text{Equation 3.10}$$

where the standard error of the difference between regression coefficients is

$$s_{b_2 - b_1} = \sqrt{\frac{\left(s^2_{Y.X}\right)_p}{\left(\sum x^2\right)_1} + \frac{\left(s^2_{Y.X}\right)_p}{\left(\sum x^2\right)_2}} \qquad \text{Equation 3.11}$$

and the pooled residual mean square is calculated as

$$\left(s^2_{Y.X}\right)_p = \frac{(residualSS)_1 + (residualSS)_2}{(residualDF)_1 + (residualDF)_2} \qquad \text{Equation 3.12}$$

where $x_i = (X_i - \overline{X})$, X = independent variable, Y = dependent variable, SS = sum of squares, DF = degrees of freedom and the subscripts 1 and 2 refers to the two regression lines being analyzed. The critical value of t for this test has (k_1-2) and (k_2-2) degrees of freedom (i.e., the sum of the two residual degrees of freedom), which is $v = k_1 + k_2 - 4$. The 1-α confidence interval for the difference between two slopes, k_2 and k_1, is

$$(k_2 - k_1) \pm t_{\alpha(2),v} s_{k_2 - k_1} \qquad \text{Equation 3.13}$$

3.7 NATURAL WATER SAMPLES

Seven different surfaces water sources were investigated as part of this study. A description of each water sample is provided in Table 3.2. The shipping time of the water samples ranged from 1 to 7 days. Once received the waters were stored in dark bottles at 4°C during the entire experimental period. The experimental period ranged from 7 days to as high as 4 months. Note that six sources were untreated raw waters while the seventh (A) was partially treated. In order to better categorize the experimental waters, each sample was submitted to an external laboratory (EnviroTest Laboratories, Edmonton, AB) for a comprehensive water quality analysis. The results of these analyses are compiled in Table A.1 of Appendix A. A summary of selected water quality parameters for each sample is provided in Table 3.3. B is the only water, which was not filtered before distribution by the concerned utility. As the information in Table 3.3 indicates, it was difficult to place these different natural waters into convenient categories. While the waters A to E were high in conductivity, total dissolved solids, alkalinity and hardness compared to waters F and G. F and G on the other hand were high in colour.

The natural waters used in this study were not assayed for the presence of oocysts prior to testing. The prepared oocysts were added to the natural waters before experiments in very high concentration (25 to 50 million into 200 mL). It was considered highly unlikely that oocysts would naturally be present in the water samples at

concentrations that even approached this order of magnitude. The expense of determining natural background oocysts concentration, therefore, was foregone.

Table 3.2: Description of the natural waters used for experiments

Water	Utility Partner	Water Source	Treatment	Time of Year Sampled
A	[a]EPCOR	North Saskatchewan River	Coagulated, Flocculated, Lime-Softened, Settled	Spring
B	Winnipeg	Shoal Lake Reservoir	None	Winter
C	Philadelphia	Schulkyll River	None	Spring
D	[b]AWWSC	Mississipi River	None	Fall
E	Calgary	Glenmore Reservoir	None	Winter
F	Port Hardy	Tsulquate River, BC	None	Spring
G	Vancouver	Seymour Watershed	None	Fall

[a]EPCOR Water Services, Edmonton, AB, Canada
[b]American Water Works Service Company, St. Louis, MO

Table 3.3: Selected measured water quality parameters for the natural waters used in the experimental trials

Parameters	Water A	Water B	Water C	Water D	Water E	Water F	Water G
PH	7.8	7.7	7.6	8.1	8.1	6.3	5.8
Conductivity (μS/cm)	294	175	604	498	496	27	16
Alkalinity (mg/L)	93	79	75	162	181	9	8
Total dissolved solids, TDS (mg/L)	171	89	348	290	279	13	9
Hardness (mg/L)	151	87	169	224	245	9	6
Total Organic Carbon, TOC (mg/L)	na	6.3	1	6	<1	4	2
Colour (TCU)	na	na	8	15	<3	30	20
Turbidity (NTU)	0.87	0.26	5.1	47	1.6	0.15	0.49

na = analysis result not available

3.8 TARGET EXPERIMENTAL CONDITIONS

Water Quality: The natural waters A, B, and C were chosen randomly without taking the water quality into consideration as they were used only for the preliminary trials. The natural waters D and E were chosen to represent two extremes of water quality (Table 3.3). Water D was collected from a river water source subject to run-off conditions during the spring and represents relatively poor quality water with high turbidity, colour and TDS. Water E was collected from a reservoir during the fall and represented relatively good quality water that was low in turbidity, colour and TDS. It is important to note that some of the parameters like conductivity, alkalinity, total dissolved solids and hardness were similar in both waters. The natural waters F and G were specifically chosen to represent low pH (6.0) natural waters. While the waters F and G were high in

colour, they were also lower in conductivity, alkalinity, TDS, pH and hardness than the other waters.

Primary Ozone Treatment: Based on the knowledge from previously published studies regarding the effect of ozone on *C. parvum* oocysts suspended in buffered de-ionized water, similar ozone *Ct* values will result in very different levels of inactivation at 1°C versus 21°C. Hence inactivation levels of 0.7 and 1.7 log-units were used to characterize primary treatment targets rather than *Ct* values. The *Ct* values were manipulated to achieve these two inactivation levels at each temperature based on the kinetic model predictions of Li et al. (2001b). In practice, the ozone demand and rate of decay were difficult to predict and reproduce in the natural waters. This made it difficult to consistently achieve the target oocyst inactivation. As a result, some deviations from the infectivity reduction targets and trial-to-trial variability in the measured infectivity reduction after the ozone treatment were encountered.

Secondary Oxidant: Inactivation of oocyts was measured after various levels of exposure to the secondary oxidant measured as the *Ct* product. In general, the experimental *Ct* values ranged from 1 000 to 5 000 mg·min/L. However, under certain circumstances, the *Ct* products greater than 2 500 mg·min/L resulted in inactivation that was beyond the detection limit of the neonatal CD-1 mouse assay. Hence, the range of experimental *Ct* products was reduced to a range of 500 to 2 500 mg·min/L for those trials. In either case, inactivation by exposure to the secondary oxidant was interpreted using the Chick-Watson rate constant, *k*, as described in Equation 3.7.

pH: The pH of the natural waters A to E, used in the experiments was restricted to a fairly narrow range of pH 7.6 to 8.1 (Table 3.3). In order to investigate the effect of pH, the pH of these water samples (A to E) in some of the trials was adjusted to a pH of 6.0 by adding concentrated sulfuric acid. The samples were then exposed to ambient air with gentle stirring overnight to ensure that the pH was stabilized at the target of 6.0. Despite this stabilization period, the pH of the experimental water was observed to slowly drift upward during the experimental time period of exposure to the secondary oxidant. This

time period sometimes extended to 16 hours. These experiments, therefore, were not conducted at a truly constant pH of 6.0. They, therefore, were identified as "initial pH 6.0". This represents an important difference from earlier experiments conducted with phosphate buffered de-ionized water in which the pH was essentially constant throughout the experiment (Li et al. 2001b). The phenomenon has important implications for the interpretation and analysis of this study. The addition of a buffer to these natural waters may have stabilized the pH; however, the original characteristics of the natural waters would have changed and hence it was not done.

The natural waters F and G had a low pH near 6.0. The pH varied little (± 0.3) during the course of the experiment for these waters and hence was considered stable at pH 6.0.

Temperature: Both high and low temperature levels were investigated. The high temperature was 21°C. During several of the low temperature trials the refrigeration unit on the water batch malfunctioned and the target temperature of 1°C could not be maintained. The actual experimental temperature was recorded as 5°C. The unit was later repaired and the 1°C temperature target was maintained in other trials.

CHAPTER 4: SEQUENTIAL INACTIVATION OF *C. PARVUM* USING OZONE FOLLOWED BY FREE CHLORINE IN NATURAL WATERS

The aim of the experiments reported in this chapter was to determine whether there was a synergistic inactivation of *C. parvum* oocysts after treatment with ozone followed by free chlorine in natural waters and to determine the important factors that might influence that synergistic effect. The experimental work was divided into three main parts (Part I, II, and III). These are as follows:

4.1 PART I: PRELIMINARY SEQUENTIAL TRIALS

4.1.1 Experimental Plan

Very little information was available about sequential inactivation trials in natural waters from previous studies. As a result it was difficult to design experiments without conducting some preliminary studies on the natural waters. Hence, Part I of this chapter describes some limited sequential trials that were conducted in 3 natural waters to better understand their behaviour under different conditions. The specific trials conducted were as follows:

1. trials with natural water A at 21°C for both high and low ozone treatment levels at the natural pH of 7.8 and with the initial pH 6;
2. trials with natural water B at 21°C and 2°C for both high and low ozone treatment levels and with the initial pH 6.0; and
3. trials with natural water C at 21°C for both high and low ozone treatment level at the natural pH of 7.6 and with the initial pH 6.0.

4.1.2 Results and Analysis

The results of the ozone followed by free chlorine sequential treatment trials with natural waters A, B, and C are shown in Figures 4.1 to 4.3. Details of the infectivity

reduction for these trials are provided in Tables B.1, B.2, and B.3 of Appendix B. Details of the ozone primary treatment conditions are provided in Tables C.1, C.2, and C.3 of Appendix C. Details of the secondary treatment conditions with free chlorine are provided in Tables D.1, D.2, and D.3 of Appendix D. In Figures 4.1 to 4.3, the total *C. parvum* infectivity reduction, measured after various levels of exposure to the free chlorine, was plotted versus the average free chlorine *Ct*. Note that each datum in these figures represents the results of a single mouse infectivity assay for a single experimental trial. The infectivity reductions of the oocysts due to ozone pretreatment alone are indicated by the data points at free chlorine $Ct = 0$.

The synergistic effect was interpreted in terms of the rate of inactivation during exposure to the free chlorine. The inactivation rate constant, k, was determined from the slope of the infectivity reduction versus free chlorine *Ct* plots. This assumes that *C. parvum* inactivation by free chlorine can be adequately described by first-order ($n = 1$) Chick-Watson type kinetics, where, the rate constant, k, is equal to the slope of the *Ct* plot (Equation 3.7). For each data set, the slope, or k values and the associated 90% confidence interval were computed, using standard least-squares linear regression techniques. If k for the sequential treatment was found to be greater than the k for the secondary treatment alone, then this was interpreted as evidence of a synergistic effect (Figure 3.1).

In a few cases, the trial result was above the upper detection limit of the infectivity assay. That is, none of the mice in the group that received the largest inoculum (typically 100 000 oocysts) became infected. These points are indicated by the > sign in the figure legends. The infectivity reductions for these data were then set at the detection limit (i.e. by assuming one mouse in the cohort was infected) and were used for the calculation of k using the least square method. Using this approach, the computed k values were biased toward a conservative interpretation of the effectiveness of the given treatment. Often these data were found to lie close to other data points for which the infectivity result was within the detection limit.

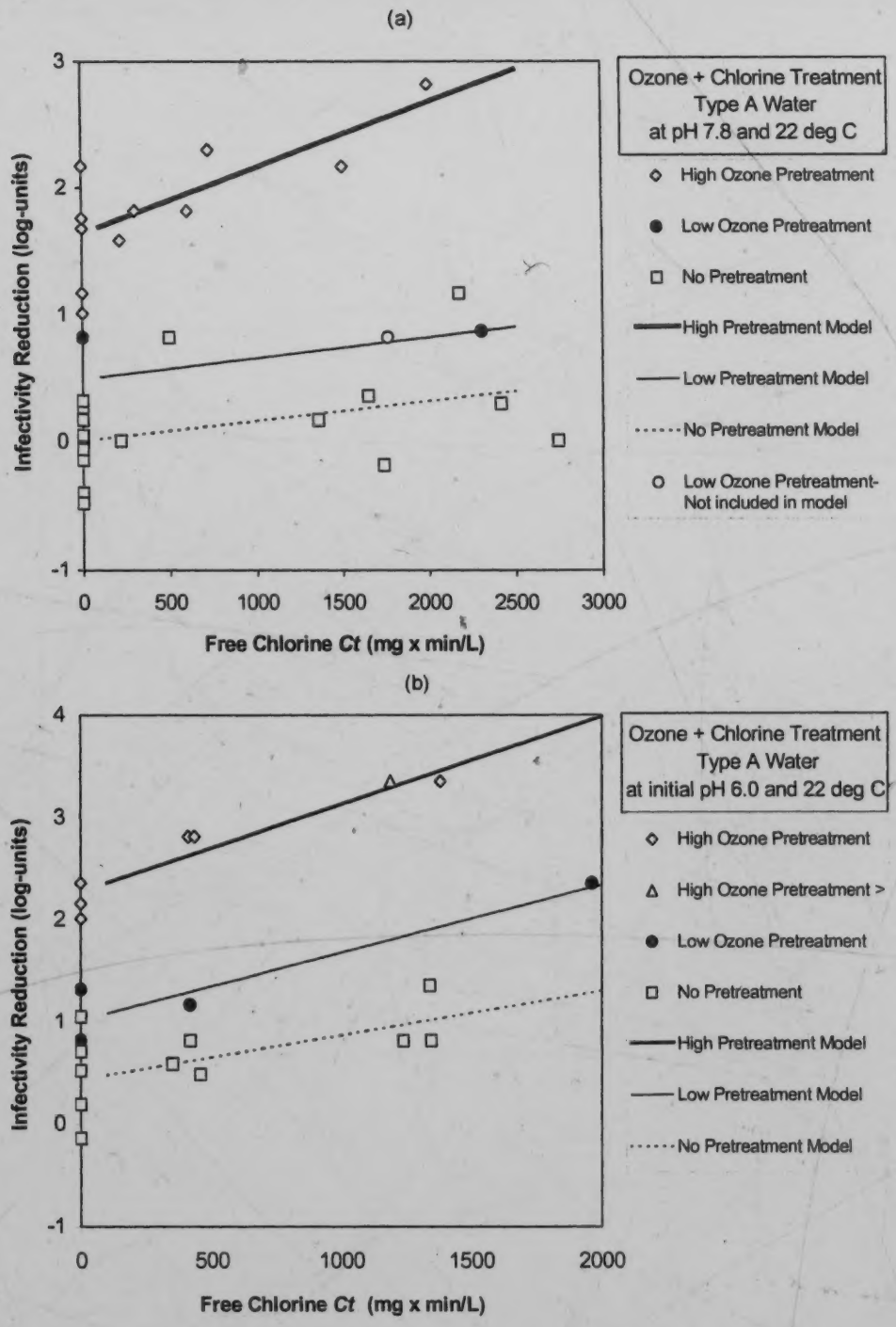

Figure 4.1 Effect of sequential treatment with ozone and free chlorine on *C. parvum* oocysts in natural water A at (a) pH 7.8 and (b) initial pH 6 at 22 °C.

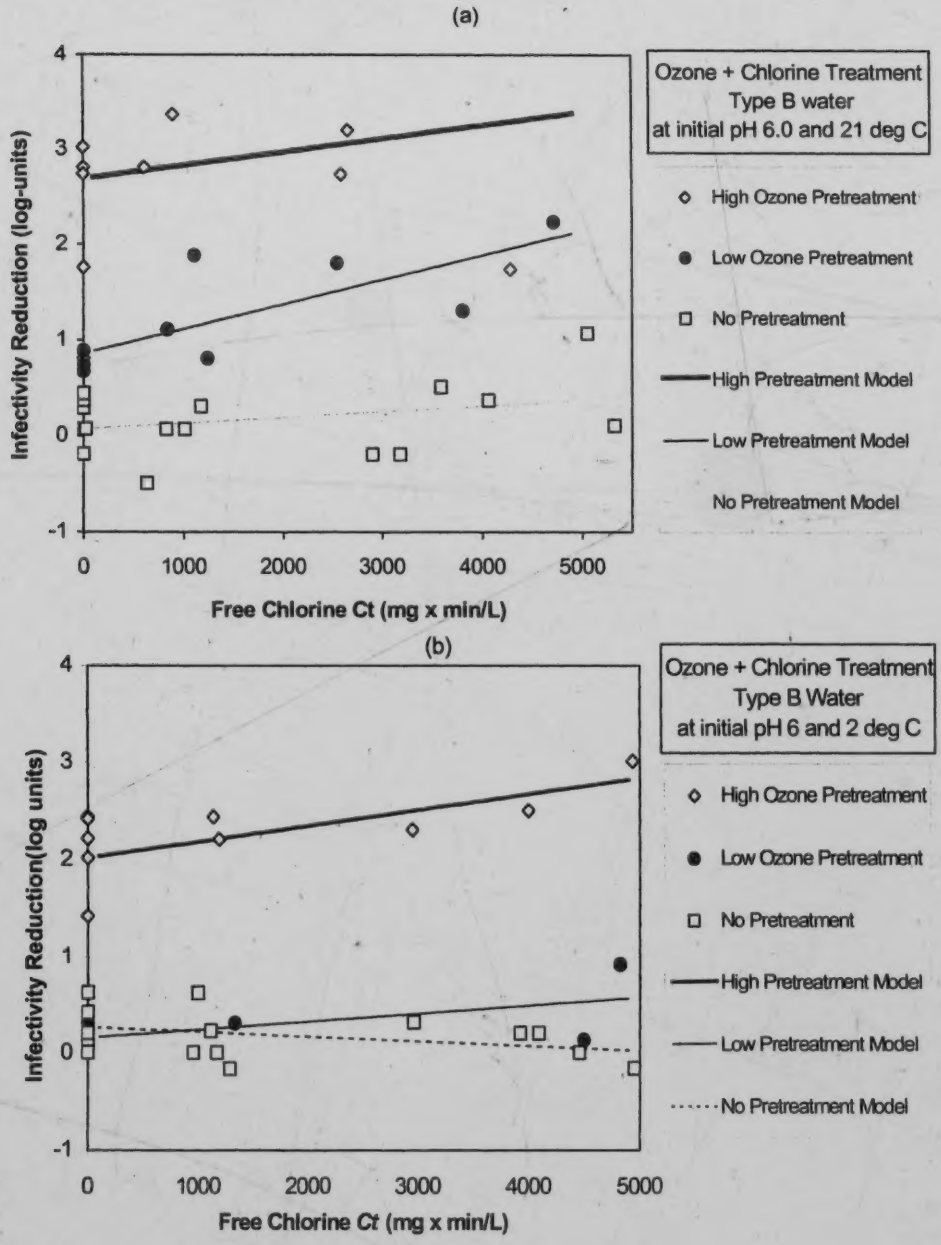

Figure 4.2 Effect of sequential treatment with ozone and free chlorine on *C. parvum* oocysts in natural water B at (a) 21 °C and (b) 2 °C at initial pH 6.

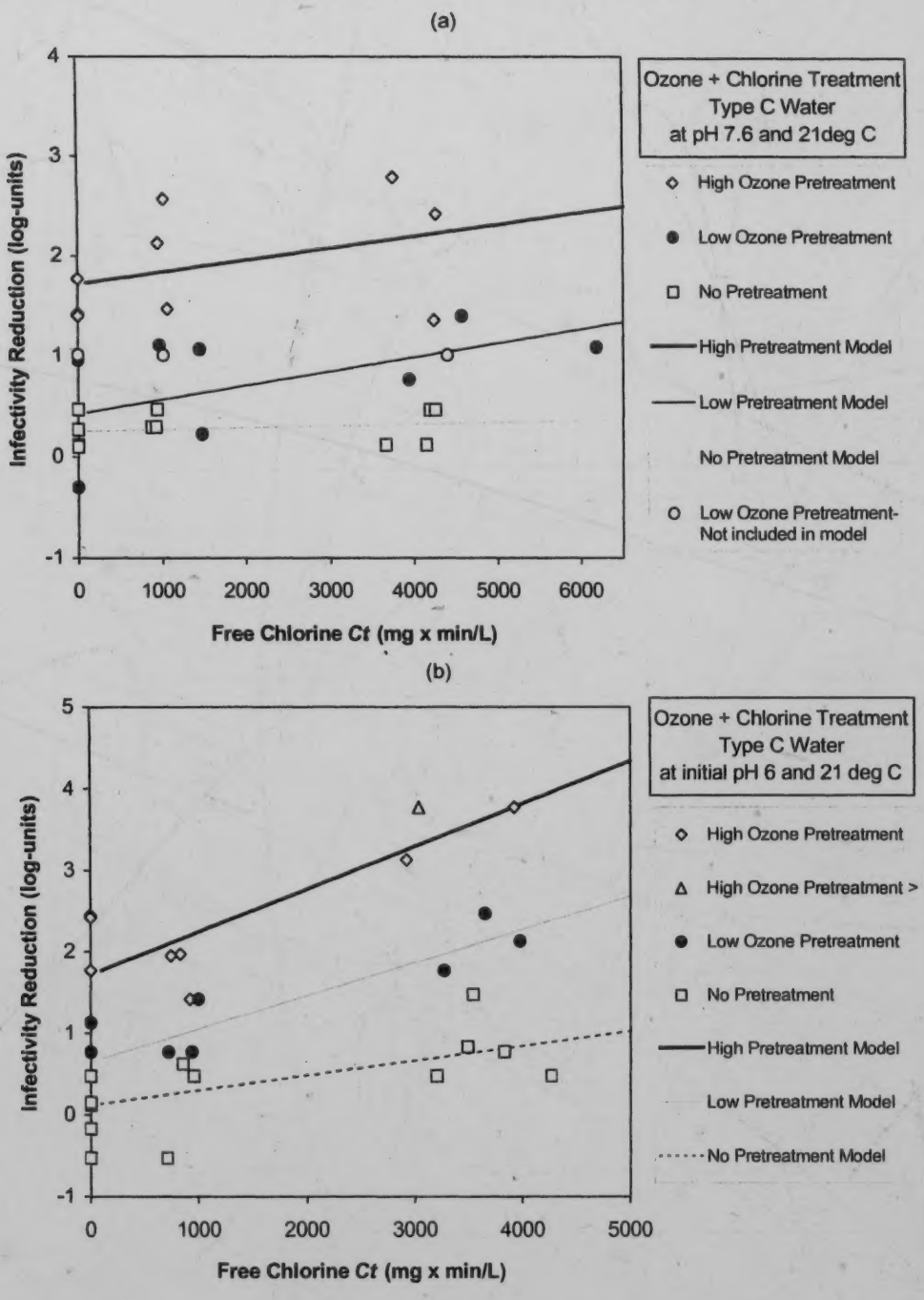

Figure 4.3 Effect of sequential treatment with ozone and free chlorine on *C. parvum* oocysts in natural water C at (a) pH 7.6 and (b) initial pH 6 at 21 °C.

In other cases, the infectivity results were less than the lower detection limit (indicated by "o" in the figure legends) of the infectivity assay. That is, all the mice in the lowest inoculum group became infected. Using these data would have tended to bias the computed k values toward a less conservative interpretation of the effectiveness of the given treatment. Therefore, below detection limit trial results shown in the figures were not used to compute the k values.

The calculated Chick-Watson rate constants, k's, for each of the trials shown in Figures 4.1 to 4.3 are provided in the Tables 4.1 to 4.3. The 90% confidence intervals on the values of k, also reported in Tables 4.1 to 4.3. If the computed 90% confidence interval on the value of k did not include zero, the computed k was determined to be statistically different from zero. The result of this test is indicated in the fifth column of Tables 4.1 to 4.3. If the computed k value (k_2) for chlorine inactivation with ozone primary treatment was greater than the computed k value (k_1) for chlorine inactivation without primary treatment, then this was considered as evidence of a synergistic effect (Figure 3.1). The difference between the slopes (k_2-k_1) was considered to be statistically significant when the level of significance (p-values) of the test statistic (Section 3.6.2 in Chapter 3) was less than 0.1. The last column in each of the tables (4.1 to 4.3) indicates if the synergistic effect measured with ozone primary treatment was statistically significant at the 90 % confidence level (p<0.1).

The results obtained with ozone and free chlorine sequential treatment (Figures 4.1 to 4.3) can be summarized as follows:

- In natural water A at pH 7.8 and 22 °C (Figure 4.1 a, Table 4.1) a synergistic effect was significant for high ozone primary treatment but was not significant for low ozone primary treatment. However, the lack of significance may have been due to the lack of sufficient data for the low ozone primary treatment condition. When the initial pH was 6.0, similar results were obtained for both high and low ozone pretreatment (Figure 4.1 b).

Table 4.1: Calculated Chick-Watson rate constants for natural water A for ozone followed by free chlorine sequential treatment at 22 °C

Figure No.	pH	Ozone Primary Treatment Level (log-units)	k ± 90 % CI[a] ($\times 10^4$ L/mg/min)	[b]Significant k	$(k_2\text{-}k_1)$ ± 90 % CI[a] ($\times 10^4$ L/mg/min)	[c]Significant Synergistic Effect? (p-values)
4.1 (a)	7.8	1.6	5.2 (2.7,7.7)	Yes	3.6 (1.2, 6.1)	Yes (0.01)
	7.8	0.49	1.7 (-11, 15)	[d]No	0.09 (-1.5, 1.7)	No (0.91)
	7.8	0	1.5 (0.35, 2.7)	Yes		
4.1 (b)	[e]6.0	2.28	8.7 (7.0, 10.4)	Yes	4.3 (2.1, 6.6)	Yes (0.004)
	[e]6.0	1.01	6.6 (2.6, 10.6)	Yes	2.3 (-0.26, 4.9)	No (0.14)
	[e]6.0	0	4.3 (1.9, 6.8)	Yes		

[a]Confidence Interval
[b]Considered significant if 90% confidence interval on the value of k do not include zero
[c]Considered significant if the p-value of the test statistic is less than or equal to 0.1
[d]Lack of significance may be due to the lack of sufficient number of data points
[e]Initial pH 6.0

- In natural water B at 21°C, there was evidence of a small synergistic effect for low ozone primary treatment when the initial pH was 6.0 (Figure 4.2 a). When the temperature was decreased to 2°C (Figure 4.2 b), there was evidence of a small synergistic effect for both high or low ozone primary treatment, though the secondary inactivation rate constants were not statistically significant (Table 4.2).

- In natural water C at the natural pH of 7.6 and at 21°C synergistic effect was evident for low ozone pre-treatment but not for high ozone pre-treatment (Figure 4.3 a, Table 4.3). When the initial pH was adjusted to 6.0 (Figure 4.3 b), statistically significant synergistic effect was observed for both high and low ozone pre-treatment (Table 4.3).

Table 4.2: Calculated Chick-Watson rate constants for natural water B for ozone followed by free chlorine sequential treatment at initial pH 6.0

Figure No.	Temp. (°C)	Ozone Primary Treatment Level (log-units)	k ± 90 % CI[a] × 10^4 L/mg/ min	[b]Significant k	(k_2-k_1) ± 90 % CI[a] (× 10^4 L/mg/min)	[c]Significant Synergistic Effect? (p-values)
4.2 (a)	21	2.7	1.4 (0.1, 2.7)	Yes	0.86 (0.12, 1.6)	Yes (0.06)
	21	0.86	2.6 (1.9, 3.3)	Yes	2 (1.4, 2.6)	Yes (0.000005)
	21	0	0.6 (0.035, 1.2)	Yes		
4.2 (b)	2	2	1.6 (-0.2, 3.4)	No	2.1 (1.5, 2.7)	Yes (0.000006)
	2	0.16	0.8 (-0.1, 1.7)	No	1.3 (0.8, 1.8)	Yes (0.0002)
	2	0	-0.5 (-0.9, -0.08)	No		

[a]Confidence Interval
[b]Considered significant if 90% confidence interval on the value of k do not include zero
[c]Considered significant if the p-value of the test statistic is less than or equal to 0.1

Table 4.3: Calculated Chick-Watson rate constants for natural water C for ozone followed by free chlorine sequential treatment at 21°C

Figure No.	pH	Ozone Primary Treatment Level (log-units)	k ± 90 % CI[a] ($\times 10^4$ L/mg/min)	[b]Significant k	(k_2-k_1) ± 90 % CI[a] ($\times 10^4$ L/mg/min)	[c]Significant Synergistic Effect? (p-values)
4.3 (a)	7.6	1.7	1.2 (-0.2, 2.6)	No	1.0 (0.22, 1.8)	Yes (0.04)
	7.6	0.43	1.4 (0.34, 2.5)	Yes	1.2 (0.6, 1.9)	Yes (0.005)
	7.6	0	0.18 (-0.17, 0.53)	No		
4.3 (b)	[d]6.0	1.7	5.2 (3.8, 6.6)	Yes	3.4 (2.4, 4.4)	Yes (0.000008)
	[d]6.0	0.65	4.1 (3.0, 5.1)	Yes	2.3 (1.4, 3.2)	Yes (0.0004)
	[d]6.0	0	1.8 (0.9, 2.6)	Yes		

[a]Confidence Interval
[b]Considered significant if 90% confidence interval on the value of k do not include zero
[c]Considered significant if the p-value of the test statistic is less than or equal to 0.1
[d]Initial pH 6.0

In general, evidence of a synergistic effect with ozone followed by free chlorine was found for each of the natural waters and for most of the experimental conditions investigated. In some cases, however, the measured synergistic effect was not statistically significant (Tables 4.1 to 4.3). This is because the magnitude of the synergistic effects was of the same order as the error associated with measurement of oocyst infectivity. Based on experience from previous studies (Gyürék et al., 1999), the typical variation in the neonatal CD-1 mouse assay is in the order of ± 0.7 log-units. For the experimental *Ct* ranges investigated, the additional level of inactivation due to the secondary treatment with chlorine, following the ozone exposure, was typically less than 1.0 log-unit. One of the important outcomes of these initial experiments was the realization that, given the experimental variability, measurement of a statistically significant synergistic effect for any given water at a selected treatment conditions would require replicate trials.

To help compare the magnitudes of the synergistic effect measured in the different waters and at the various treatment conditions, *Ct* products required for a 1 log-unit synergistic effect were estimated according to:

$$Ct_{1\text{-log}} = \frac{1}{k_2 - k_1} \qquad \text{Equation 4.1}$$

Here k_1 represents the rate constant for the secondary oxidant alone and k_2 represents the rate constant for the secondary oxidant after ozone primary treatment (i.e. sequential). Values of k_1 and k_2 used in the computation of $Ct_{1\text{-log}}$ were taken from Tables 4.1 to 4.3. The *Ct* products required for 1 log-unit synergistic effect thus estimated are compiled in Table 4.4. The last column of Table 4.4 indicates whether "k_2-k_1" for each test condition was statistically significant when tested at the 90 % confidence level. A statistically significant synergistic effect was observed in most of the trials. However, their magnitude was small and variable. In addition, a wide range of $Ct_{1\text{-log}}$ product was calculated. No obvious trends or relationships between the magnitude of the synergistic effect and experimental variables such as temperature and water quality are evident from the information in Table 4.4.

Table 4.4: Estimated free chlorine *Ct* products required for a 1 log-unit synergistic effect in the natural waters A, B, and C for ozone followed by chlorine sequential treatment

Water	Test Conditions	Ozone Pre-Treatment Level (log-units)	*Ct* Required for 1 Log-Unit Synergistic Effect (mg×min/L)	Statistically significant?
A	21°C, pH 8.5	1.6	2 700	Yes
A	21°C, pH 8.5	0.49	Too large to measure	No
A	21°C, pH 6.0[a]	2.3	2 300	Yes
A	21°C, pH 6.0[a]	1.01	4 300	No
B	21°C, pH 6.0[a]	2.7	11 600	Yes
B	21°C, pH 6.0[a]	0.86	5 000	Yes
B	2°C, pH 6.0[a]	2	4 700	Yes
B	2°C, pH 6.0[a]	0.16	7 500	Yes
C	21°C, pH 6.0[a]	1.7	2 900	Yes
C	21°C, pH 6.0[a]	0.65	4 400	Yes
C	21°C, pH 7.6	1.7	9 900	Yes
C	21°C, pH 7.6	0.43	8 100	Yes

[a]Initial pH 6.0

4.2 PART II: FACTORIAL DESIGNED EXPERIMENTS

After the initial phase of this study, the experimental plan was refined. Based on the experience gained in Part I the level of variability in the outcomes of the animal infectivity assays, relative to the magnitude of the synergistic effect, was identified as a limiting factor for the reliable determination of a synergistic effect. It was concluded that the measurement of a synergistic effect with a satisfactory degree of statistical confidence required replicate experimental trials. Therefore in Part II of this study, a modified experimental approach for the determination of a synergistic effect on *C. parvum* inactivation in the natural waters was developed. This procedure called for triplicate trials at each experimental condition with separate measurements of inactivation after ozone primary treatment, and after two different levels of secondary *Ct* product for each replicate (Figure 3.1). This was complemented by triplicate trials of the secondary treatment with no ozone primary treatment.

Using the new procedure for measurement of the synergistic effect, a factorial experimental design was developed using two additional natural water samples. The primary objective of the factorial design was to determine if the following experimental variables had an effect on the magnitude of the measured synergistic effect:

1. pH;
2. temperature;
3. ozone primary treatment level; and
4. water quality.

4.2.1 Experimental Plan

The levels of the experimental factors that were selected for investigation and the design matrix are described in Table 4.5. In this table, the levels of the experimental factors are scaled from −1 to +1. The four factors were addressed in a 2^{4-1} fractional

Table 4.5: The 2^{4-1} design matrix for ozone followed by free chlorine sequential treatment

Factors		Experimental Factor Targets	
		-1	1
1	Natural Water	D	E
2	Ozone Pre-treatment Level	Low (0.7 log-unit)	High (1.7 log-unit)
3	pH	Initial pH 6.0	8.1
4	Temperature (°C)	1	21

Trial No.	[a]Factor Levels in Scaled-Units				No. of Replicates
	1	2	3	[b]4=123	
1	1	1	1	1	3
2	-1	1	1	-1	3
3	1	-1	1	-1	3
4	-1	-1	1	1	3
5	1	1	-1	-1	3
6	-1	1	-1	1	3
7	1	-1	-1	1	3
8	-1	-1	-1	-1	3

[a]Identification of the factors is provided at the top of the table
[b]Dummy factor used to generate the fractional factorial design.

design that consisted of a total of eight experimental conditions. The factorial design approach was chosen in order to investigate a larger number of variables while keeping the total number of experimental trials required to an acceptable level. A half-fraction design such as this permits all the main effects to be determined free of interactions with other main effects or two factor interactions. It also permits two factor interactions to be determined if higher order interactions are assumed to be negligible (Box and Hunter, 1978). To address the variability inherent in the mouse infectivity assay, and to permit statistical interpretation of the results, each experimental condition was replicated twice (i.e. three experimental trials for each experimental condition).

4.2.2 Results and Analysis

Figures 4.4 to 4.7 show the results of experiments completed at the various experimental conditions specified by the fractional factorial design matrix (Table 4.5) for the sequential trials with ozone followed by free chlorine. Details of the infectivity reduction for these trials are provided in Tables B.4 and B.5 of Appendix B. Details of the ozone primary treatment conditions are provided in Tables C.4 and C.5 of Appendix C. Details of the secondary treatment conditions with free chlorine are provided in Tables D.4 and D.5 of Appendix D. The data sets in Figures 4.4 to 4.7 were modeled using the first-order (n = 1) Chick-Watson analysis (Equation 3.7), and the first-order Chick-Watson rate constants, k, were calculated using least-squares linear regression. The outcomes of triplicate trials are also shown in the figures as individual data points. That is, each datum represents a single infectivity analysis result. In many cases it was difficult to replicate the Ct condition exactly.

The data points in Figures 4.4 and 4.6 for which the infectivity assay were unusually higher or lower than the detection limit (indicated by "o" in the figure legends) were not used for calculating k. For example, the above detection limit data points (0, 2.6), (615, 3.3) and (1467, 3.9) in Figure 4.4 (b), were not used for calculating k. This is because these three data points were generated in the same sequential treatment trial in which the inactivation due to ozone alone was unusually high. However, depending on the circumstances, certain data points (indicated by > in the figure legends) for which the infectivity analysis was greater than the upper detection limit were set at the detection limit of the data points and were used in computation of the k (Figure 4.6 b).

The computed rate constants and associated 90% confidence intervals are provided in Tables 4.6 and 4.7. A small synergistic effect was evident at the lower temperature for water D and at the higher temperature for water E (Table 4.6 and 4.7). However, in general, there was no evidence of a synergistic effect with ozone followed by free chlorine sequential treatment for the waters D and E.

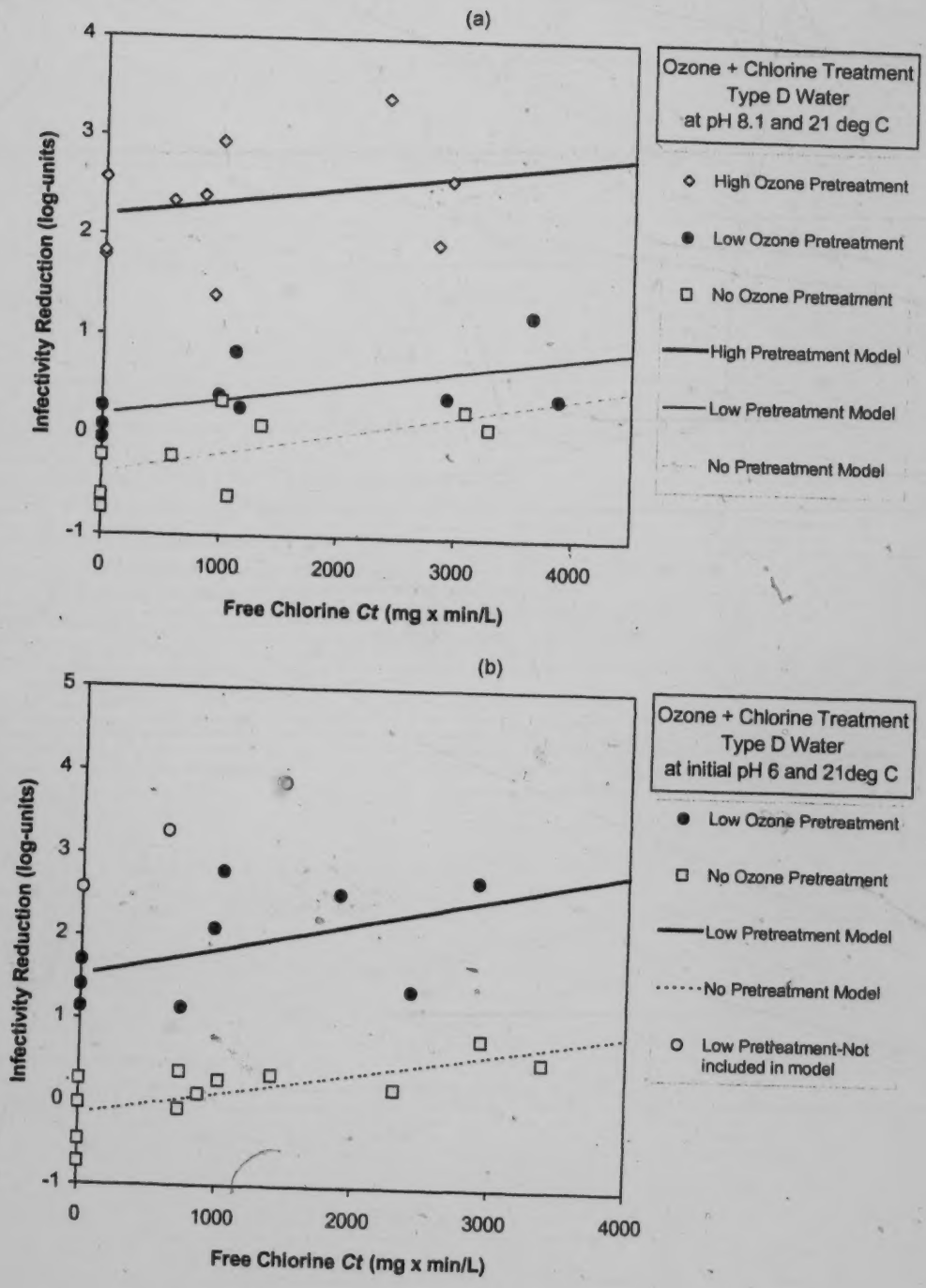

Figure 4.4 Effect of ozone and free chlorine treatment on *C. parvum* oocysts in natural water D at (a) pH 8.1 and (b) initial pH 6 at 21 °C.

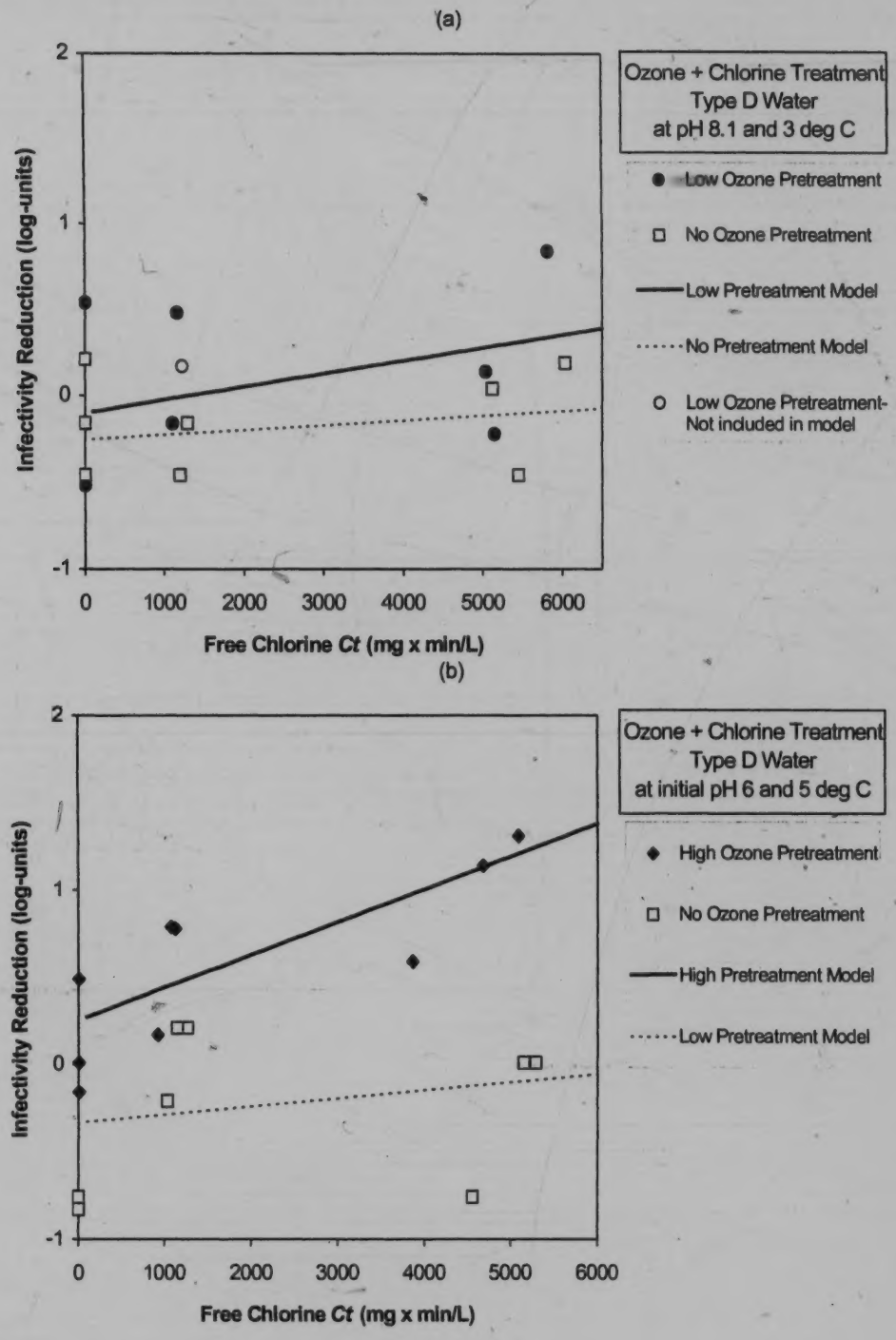

Figure 4.5 Effect of ozone and free chlorine treatment on *C. parvum* oocysts in natural water D at (a) pH 8.1, 3 °C and (b) initial pH 6 at 5 °C.

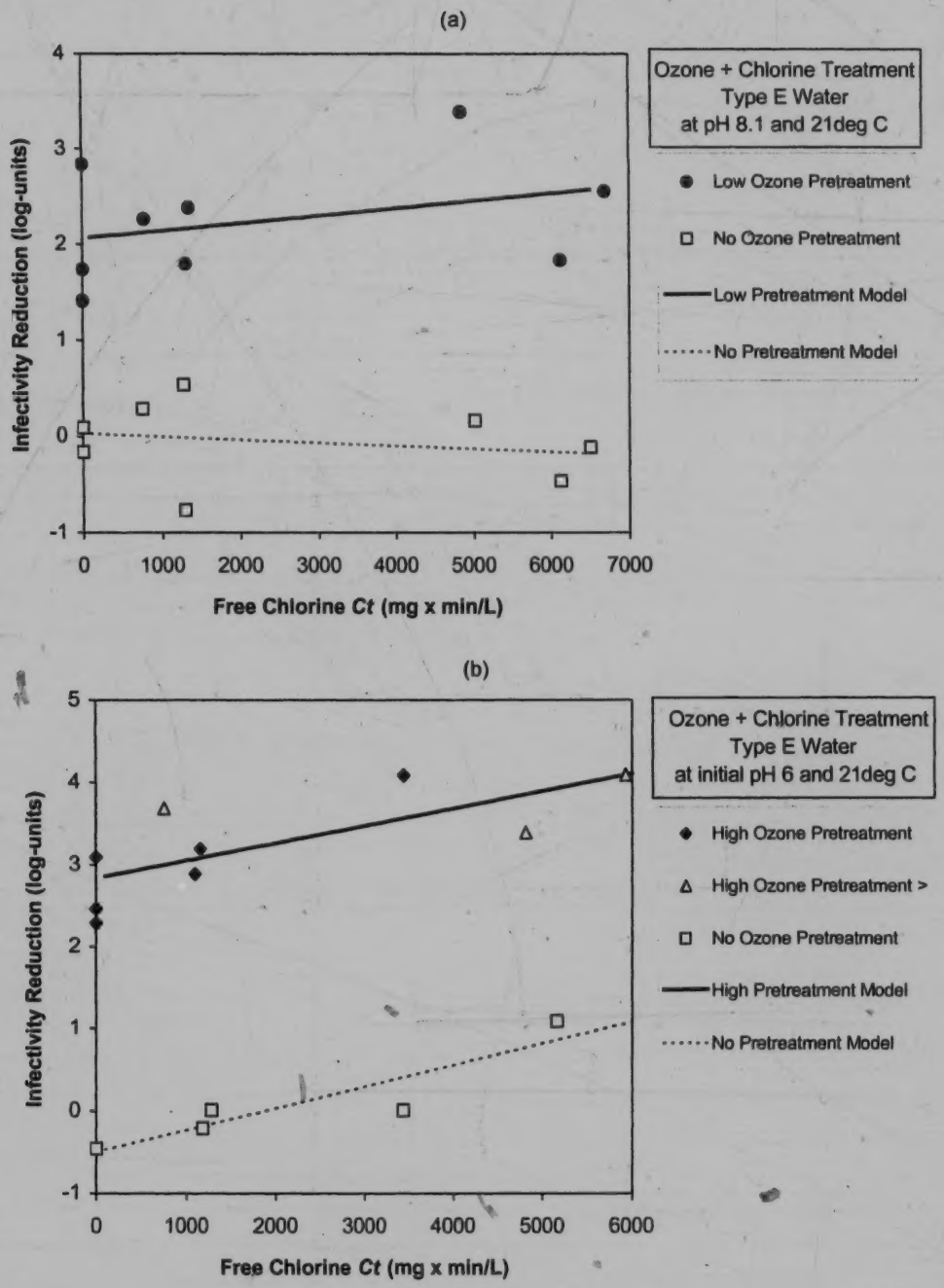

Figure 4.6 Effect of ozone and free chlorine treatment on *C. parvum* oocysts in natural water E at (a) pH 8.1 and (b) initial pH 6 at 21 °C.

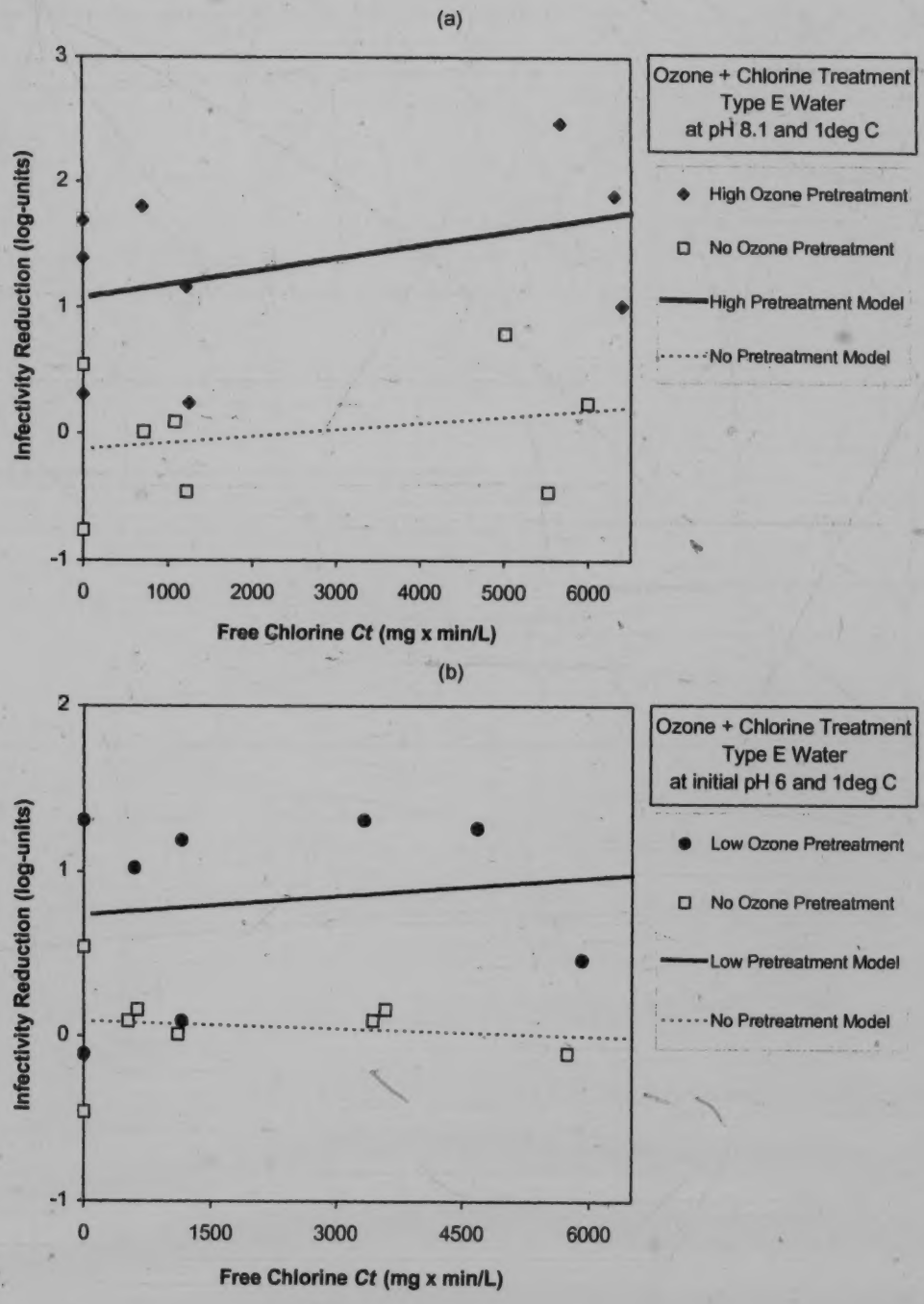

Figure 4.7 Effect of ozone and free chlorine treatment on *C. parvum* oocysts in natural water E at (a) pH 8.1 and (b) initial pH 6 at 1 °C.

Table 4.6: Calculated Chick-Watson rate constants for ozone followed by free chlorine sequential trials with natural water D

Fig. No.	pH	Temp (°C)	Ozone Primary Treatment Level (log-units)	k ± 90 % CI[a] ($\times 10^4$ L/mg/min)	[b]Significant k?	(k_2-k_1) ± 90 % CI[a] ($\times 10^4$ L/mg/min)	[c]Significant Synergistic Effect? (p-values)
4.4 (a)	8.1	21	2.2	1.4 (-0.7, 3.5)	No	-0.54 (-2.1, 1.1)	No (0.65)
	8.1	21	0.2	1.5 (0.54, 2.5)	Yes	-0.43 (-1.4, 0.54)	No (0.44)
	8.1	21	0	1.9 (0.78, 3.1)	Yes		
4.4 (b)	[d]6.0	21	1.5	3.2 (0.7, 5.7)	Yes	0.71 (-0.86, 2.3)	No (0.44)
	[d]6.0	21	0	2.8 (1.4, 4.2)	Yes		
4.5 (a)	8.1	3	-0.1	0.75 (-0.3, 1.8)	No	0.48 (-0.27, 1.2)	No (0.28)
	8.1	3	0	0.28 (-0.3, 0.85)	No		
4.5 (b)	[d]6.0	5	0.25	1.9 (1.2, 2.5)	Yes	1.4 (0.65, 2.2)	Yes (0.0056)
	[d]6.0	5	0	0.46 (-0.5, 1.4)	No		

[a]Confidence Interval
[b]Considered significant if 90% confidence interval on the value of k do not include zero
[c]Considered significant if the p-value of the test statistic is less than or equal to 0.1
[d]Initial pH of 6.0

Table 4.7: Calculated Chick-Watson rate constants for ozone followed by free chlorine sequential trials with natural water E

Fig. No.	pH	Temp. (°C)	Ozone Primary treatment Level (log-units)	$k \pm 90\%$ CI[a] ($\times 10^4$ L/mg/min)	[b]Significant k?	$(k_2-k_1) \pm 90\%$ CI[a] ($\times 10^4$ L/mg/min)	[c]Significant Synergistic Effect? (p-values)
4.6 (a)	8	21	2.1	0.78 (-0.32, 1.9)	No	1.1 (0.17, 2.0)	Yes (0.056)
	8	21	0	-0.32 (-0.5, -1.1)	Yes		
4.7 (a)	8	1	1.1	1.05 (-0.21, 2.3)	No	0.53 (-1.1, 2.1)	No (0.57)
	8	1	0	0.52 (-0.6, 1.6)	No		
4.6 (b)	[d]6.0	21	2.8	2.1 (1.1, 3.1)	Yes	-0.5 (-1.5, 0.5)	No (0.39)
	[d]6.0	21	0	2.6 (1.6, 3.6)	Yes		
4.7 (b)	[d]6.0	1	0.74	0.37 (-1.0, 1.8)	No	0.53 (-0.55,1.6)	No (0.4)
	[d]6.0	1	0	-0.16 (-0.92, 0.6)	No		

[a]Confidence Interval
[b]Considered significant if 90% confidence interval on the value of k do not include zero
[c]Considered significant if the p-value of the test statistic is less than or equal to 0.1
[d]Initial pH 6.0

4.2.3 Statistical Analysis

The objective of the factorial design was to determine which experimental variables had a statistically significant impact on the measured synergistic effect. The results of the factorial designed experiment for ozone followed by free chlorine sequential treatment are summarized in Table 4.8. The synergistic effect is represented as the difference between the rate of inactivation induced by the secondary treatment after primary treatment (k_2) and without primary treatment (k_1). In the tables this is given by the value of "k_2-k_1". Even though the measured synergistic effect was not statistically significant in a number of the experimental trials, these results were still used in the foregoing statistical analysis. Although several of the individual determinations of

Table 4.8: Summary of synergistic effect determinations for the factorial design experiments with ozone followed by free chlorine sequential treatment

Result No.	Natural Water	Synergistic Effect k_2-k_1 ($\times 10^4$ L/mg/min)	Ozone Primary Treatment (log-units)	Temperature (°C)	pH
1	D	-0.54	2.2	21	8
2	D	-0.43	0.2	21	8
3	D	0.71	1.5	21	6
4	D	0.48	-0.1	3	8
5	D	1.4	0.25	5	6
6	E	1.1	2.1	21	8
7	E	-0.5	2.8	21	6
8	E	0.53	1.1	1	8
9	E	0.53	0.74	1	6

synergy were found to be statistically insignificant, the measured values were incorporated into the statistical analysis as elimination of these measurements of synergy or setting the values to zero would have reduced the power of the statistical analysis and would have tended to introduce bias into the statistical analysis. The statistical resolving power of the pooled datasets will be much greater than the individual measurements of synergistic effect because it comprises a much larger number of infectivity measurements. Assuming that the standard deviation of the infectivity measurements is constant, the standard error of the pooled data set will be much smaller than that of the individual measurements of synergistic effect. This is similar to the pooling of data sets for common statistical operations such as the Analysis of Variance (ANOVA).

The results summarized in Table 4.8 were analyzed using multiple linear regression to determine which experimental factors had a significant impact on the synergistic effect. To facilitate the multiple linear regression analysis, the levels of the experimental factors in Table 4.8 were first scaled so that the levels of each ranged from approximately –1 to +1. The scaling is described in Table 4.9. Using the scaled values of the variables a multi-linear model of the form of equation 3.9 was regressed to the outcomes. The dependent variable in equation 3.9, Y represented the magnitude of the synergistic effect, k_2-k_1, and the independent variables X_1, X_2, X_3, and X_4 represented ozone primary treatment level, temperature, pH, and water quality, respectively. Regression analysis was done using the regression tool in Microsoft Excel 98 described earlier (Draper and Smith, 1966).

The results of the regression analysis indicated that for the ozone and free chlorine sequential trials (Table 4.8), none of the experimental factors were statistically significant at the 90% confidence level. The p-values for each of the computed parameters, a_i, were greater than 0.10 and Equation 3.9 was reduced to:

$$\hat{Y} = 0.42 \qquad \text{Equation 4.2}$$

Table 4.9: Results from the factorial design experiment of the sequential trials using ozone and free chlorine sequential treatment represented in terms of scaled variables

Factors	-1	1
Ozone Primary Treatment Level (log units)	0.7	1.7
Temp. (°C)	1	21
pH	Initial pH 6.0	8.1
Natural Water	D	E

Result No.	Synergistic Effect (Y)	Scaled Experimental Variables			
		Ozone Primary Treatment Level (X_1)	Temp. (X_2)	pH (X_3)	Water Quality (X_4)
1	-0.54	2	1	1	-1
2	-0.43	-2	1	1	-1
3	0.71	0.6	1	-1	-1
4	0.48	-2.6	-0.8	1	-1
5	1.4	-1.9	-0.6	-1	-1
6	1.1	1.8	1	1	1
7	-0.5	3.2	1	-1	1
8	0.53	-0.2	-1	1	1
9	0.53	-0.92	-1	-1	1

Equation 4.2 implies that, within the experimental ranges investigated, the synergistic effect was unaffected by the experimental variables and was constant at an average value 0.42.

The computed 90% confidence interval on the average value was 0.01 to 0.86. This synergistic effect was, therefore, statistically significant. Using equation 4.1, this synergistic effect implies that on average a free chlorine *Ct* of 23 800 mg·min/L was required to achieve 1 log-unit of synergistic effect.

It is very likely that the synergistic effect was actually influenced by some of the experimental variables to some degree. But it is possible that the effect of the variables may have been too small to be measurable relative to the variation inherent in the infectivity assay.

It appears that for the two natural waters, chlorine seems to be ineffective as a secondary oxidant since a synergistic effect was absent in most of the sequential trials. Although the experimental conditions used for each secondary oxidant differed somewhat, partly due to the nature of the fractional factorial experimental design and partly due to practical experimental limitations, there was considerable overlap (see Tables 4.6 and 4.7). One of the limitations of the preceding regression analysis is that it was not possible to investigate the effect of individual water quality characteristics because only two waters were investigated in the factorial designed experiments.

To facilitate a comparison between the magnitudes of the synergistic effect measured in the different waters at the various treatment conditions, the *Ct* products required for a 1 log-unit synergistic effect were estimated using equation 4.1. The calculated *Ct* products are compiled in Table 4.10. The last columns of Table 4.10 indicate whether "k_2-k_1" for each test condition was statistically significant when tested at the 90 % confidence level. The very large $Ct_{1\text{-log}}$ values in Table 4.10 clearly indicate the ineffectiveness of sequential treatment with ozone and free chlorine for generating a synergistic effect with either the high quality (E) or low quality (D) water. In some cases it was not possible to calculate a meaningful synergistic effect because the value of k_2 was less than the value of k_1. The high *Ct* products required for 1 log-unit synergistic effect for waters D and E are consistent with the findings for the A, B, and C waters.

Table 4.10: Estimated free chlorine *Ct* products required for a 1 log-unit synergistic effect for ozone followed by free chlorine sequential trials in the designed experiment

Natural Water	Water Conditions	Ozone Primary Treatment Level (log-units)	*Ct* Required for 1 log-unit synergistic Effect (mg×min/L)	Statistically significant?
D	21°C, pH 8.1	2.2	Too large to measure	No
D	21°C, pH 8.1	0.2	Too large to measure	No
D	21°C, pH 6.0[a]	1.5	25 000	No
D	3°C, pH 8.1	-0.1	21 000	No
D	5°C, pH 6.0[a]	0.25	6 900	Yes
E	21°C, pH 8.1	2.1	12 800	Yes
E	21°C, pH 6.0[a]	2.8	Too large to measure	No
E	1°C, pH 8.1	1.1	18 800	No
E	1°C, pH 6.0[a]	0.74	27 000	No

[a]Initial pH 6.0

4.3 PART III: LOW PH NATURAL WATER EXPERIMENTS

The trials in Part III were conducted after the experiments of Part I and II when it was realized that one of the major limitations in all the previous trials was the inability to conduct the experiments in natural waters at a stable pH of 6.0. In order to overcome the problem, a set of additional trials was done with natural waters having a low pH near about 6.0. The trials helped us to understand whether pH was responsible for the lack of synergistic effect of *C. parvum* in natural waters for ozone followed by free chlorine sequential treatment.

4.3.1 Experimental Plan

The experimental plan was to conduct a set of sequential trials with two low pH (6.0) natural waters (Type F and G) using ozone followed by free chlorine sequential treatment. The specific sequential trials done were as follows:

1. ozone followed by free chlorine sequential treatment at low ozone pretreatment (0.7 log units inactivation) at 21°C and pH 6.3 in Type F water;
2. ozone followed by free chlorine sequential treatment at high ozone pretreatment (1.7 log units inactivation) at 5°C and pH 6.3 in Type F water;
3. ozone followed by free chlorine sequential treatment at high ozone pretreatment (1.7 log units inactivation) at 5°C and pH 5.8 in Type G water.

4.3.2 Results and Analysis

Figures 4.8 and 4.9 show the results of experiments conducted with ozone followed by free chlorine sequential treatment in low pH natural waters (Types F and G). Details of the infectivity reduction for these trials are provided in Tables B.6 and B.7 of Appendix B. Details of the ozone primary treatment conditions are provided in Tables C.6 and C.7 of Appendix C. Details of the secondary treatment conditions with free

chlorine are provided in Tables D.6 and D.7 of Appendix D. Similar to Parts I and II, the data sets in Figures 4.8 and 4.9 were modeled using the first-order (n=1) Chick-Watson analysis (Equation 3.7) and the first-order Chick-Watson rate constants, k, were calculated using linear least squares regression.

The data point in Figure 4.8 (indicated by > in the figure legend) for which the infectivity analysis was greater than the upper detection limit were set at the detection limit of the data points and were used in the computation of "k". The computed rate constants and associated 90 % confidence intervals are provided in Table 4.11. There was a statistically significant synergistic effect observed for both Types F and G water under the studied conditions.

To facilitate a comparison between the magnitudes of the synergistic effect measured in the two waters at the various treatment conditions, the Ct products required for a 1 log-unit synergistic effect were estimated using equation 4.1. The calculated Ct products are compiled in Table 4.12. The last column of Table 4.12, indicate whether "k_2-k_1" for each test condition was statistically significant when tested at the 90 % confidence level. Although the synergistic effect was statistically significant at the 90% confidence level in only 1 of the 3 experiments, the measured synergistic effects were between 3 and 16 times greater than the mean synergistic effect measured in the part I and part II experiments. For natural water F at 21°C, pH 6.3 and an ozone primary treatment equivalent to 0.8 log-unit the synergistic effect was highly significant statistically (p = 0.004). In fact, in this experiment the infectivity reduction at the highest chlorine $C_{avg}t$ value tested (2 100 mg·min/L) was beyond the detection limit of the neonatal CD-1 mouse assay; that is none of the mice was positive for infection (Figure 4.8 a). In this case, the value of k_2 was computed by setting the value of the log survival ratio to the detection limit of 3.1 for this datum. The true synergistic effect may, therefore, have been greater than the computed value of 91 x 10^{-5} L/mg/min.

One of the key observations in Table 4.12 was that the Ct required for 1 log-unit synergistic effect, was very low compared to the Ct requirements for almost all the previous (A to E) waters at pH 8. This indicates that the synergistic effect was higher in waters F and G compared to the synergistic effect observed in waters A to E.

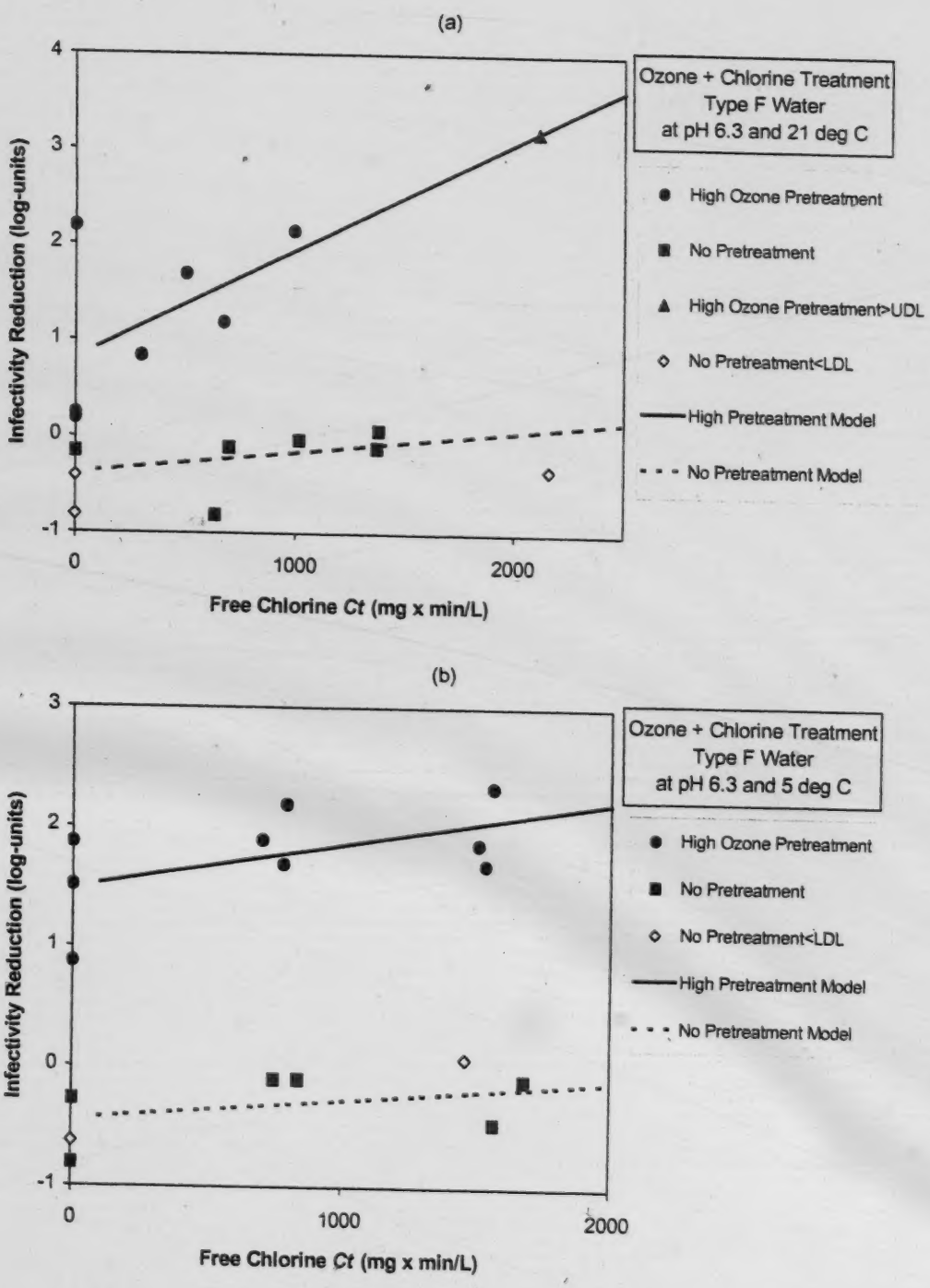

Figure 4.8 Effect of ozone and free chlorine sequential treatment on *C. parvum* oocysts in natural water F at (a) 21 °C and (b) 5 °C at pH 6.3.

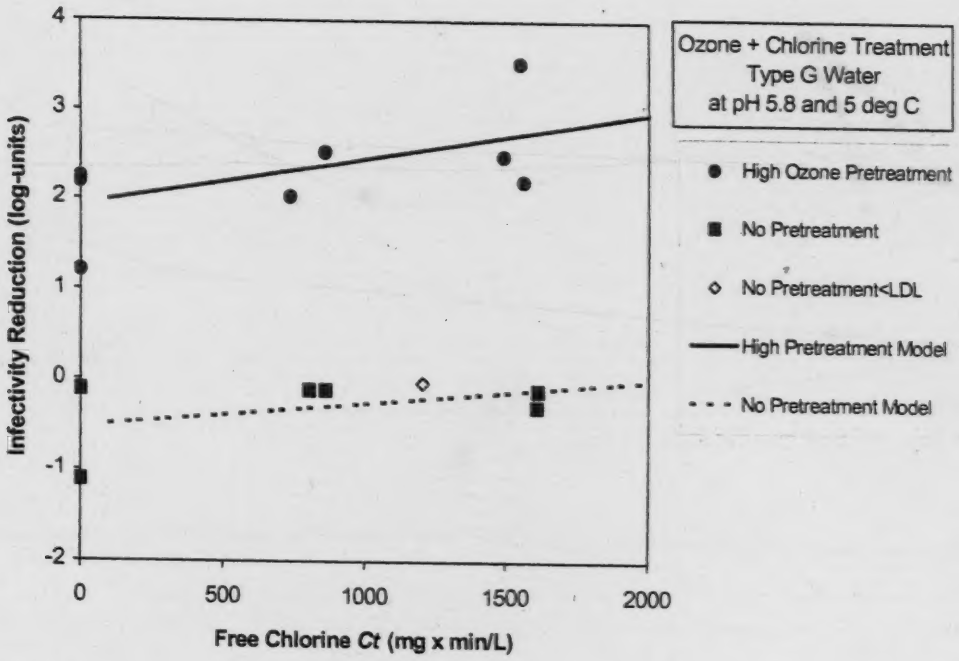

Figure 4.9 Effect of ozone and chlorine sequential treatment on *C. parvum* oocysts in natural water G at pH 5.8 and 5 °C.

Table 4.11: Calculated Chick-Watson rate constants for ozone followed by free chlorine sequential trials with natural waters F and G

Figure No.	pH	Temp. (°C)	Ozone Primary Treatment Level (log-units)	$k \pm 90\%$ CI[a] ($\times 10^4$ L/mg/min)	[b]Significant k?	$(k_2-k_1) \pm 90\%$ CI[a] ($\times 10^4$ L/mg/min)	[c]Significant Synergistic Effect? (p-values)
4.8 (a)	6.3	21	0.81	11.3 (5.8, 16.8)	Yes	9.1 (4.8, 13.4)	Yes (0.0035)
	6.3	21	0	2.2 (-0.7, 5.1)	No		
4.8 (b)	6.3	5	1.5	3.6 (1.2, 5.9)	Yes	1.99 (-0.24, 4.2)	No (0.14)
	6.3	5	0	1.6 (-0.8, 4.0)	No		
4.9	5.8	5	1.9	5.1 (1.8, 8.4)	Yes	2.5 (-0.57, 5.7)	No (0.17)
	5.8	5	0	2.6 (-0.7, 5.9)	No		

[a]Confidence Interval
[b]Considered significant if 90% confidence interval on the value of k do not include zero
[c]Considered significant if the p-value of the test statistic is less than or equal to 0.1

Table 4.12: Estimated free chlorine *Ct* products required for a 1 log-unit synergistic effect for ozone followed by free chlorine sequential trials in Types F and G water

Natural Water	Water Conditions	Ozone Primary Treatment Level (log-units)	*Ct* Required for 1 log-unit Synergistic Effect (mg×min/L)	Statistically significant?
F	21°C, pH 6.3	0.81	1 100	Yes
F	5°C, pH 6.3	1.5	5 000	No
G	5°C, pH 5.8	1.9	4 000	No

4.4 CONTROL TRIALS

The reduction in infectivity in the control trials of all the sequential trials with ozone followed by free chlorine, ranged from –0.8 to 1.0 log-unit. This range was slightly greater than the –0.7 log-unit to 0.7 log-unit range that is typical for untreated control samples when using the neonatal CD-1 mouse assay. Histograms showing the reduction in infectivity of the oocysts in the control reactors for each natural water, are presented in Appendix E. The mean inactivation in the positive controls of all the sequential trials with ozone followed by free chlorine was 0.1 log-unit indicating that exposure to the experimental water matrices and the various processing steps had little effect on mean oocyst infectivity. This also suggests that exposure of the oocysts to natural waters alone had little effect on oocyst infectivity, but may have increased the variability in the infectivity assay. The control trials served primarily as a quality assurance for the experiments.

Table 4.13: Comparison of *Ct* required for 1 log-unit synergistic effect between buffered de-ionized water and natural waters for ozone followed by free chlorine sequential treatment

Natural Water Conditions	Buffered Water Conditions	[a]Ozone Pre-Treatment (log-units)	$Ct_{1\text{-log}}$ (mg×min/L)	
			Natural Water	[b]Buffered Water
21°C at initial pH 6.0	21°C at pH 6.0	0.4	[c]23 800	560
1°C at initial pH 6.0	1°C at pH 6.0	0.4	[c]23 800	8 700
21°C at initial pH 6.0	21°C at pH 6.0	1.6	[c]23 800	710
1°C at initial pH 6.0	1°C at pH 6.0	1.6	[c]23 800	2 300
21°C at pH 6.3 (Water F)	21°C at pH 6.0	0.8	[d]1 100	Not available
5°C at pH 6.3 (Water F)	5°C at pH 6.0	1.5	[d]5 000	Not available
5°C at pH 5.8 (Water G)	5°C at pH 6.0	1.9	[e]4 000	Not available

[a]These are ozone primary treatment targets and were not necessarily achieved in individual experiments
[b]Model predictions of Li et al. (2001b) calculated using Equation 4.1 of this study
[c]Model predictions in this study calculated using Equations 4.1 and 4.2
[d]Calculated from the results of low pH natural water F using Equation 4.1
[e]Calculated from the results of low pH natural water G using Equation 4.1

4.5 DISCUSSION

The study with ozone followed by free chlorine sequential treatment in the different natural waters was conducted mainly to determine the extent of synergistic effect and the influencing factors. Among all the previous studies for determining the synergistic effect using ozone followed by free chlorine, the more comprehensive were the ones conducted by Li et al (2001b). More importantly these researchers used very similar protocols as this study including a quantitative mouse infectivity assay to determine the inactivation kinetics of *C. parvum*-ozone system and used them to develop engineering design and performance criteria. However, all those studies were conducted in buffered de-ionized water. Nevertheless, a comparison of the studies with buffered de-ionized water and natural water of this study will help us to find whether the model and design criteria developed in buffered de-ionized water is applicable in natural waters. In Table 4.13, the model predictions of the free chlorine *Ct* products required for 1 log-unit of synergistic effect are summarized using the information from this study with natural waters and the information from the study done by Li et al. (2001b) with buffered de-ionized water. For the natural waters, the predictive equations 4.1 and 4.2 were used to generate the estimates of synergistic effect. Results of the experiments with natural waters F and G were also included for comparison purposes.

4.5.1 Effect of pH

For ozone followed by free chlorine sequential trials in natural waters, the results obtained were categorized into two groups: one with higher pH (waters A to E with pH 8) and the other with lower pH (waters F and G with pH 6). In the high pH waters (Table 4.13), the measured synergistic effect was strongly inhibited and was significantly less compared to that of the buffered de-ionized water from the earlier study of Li et al. (2001b). The magnitude of the synergistic effect determined in the high pH natural water samples was between 3 and 42 times smaller than previously reported for buffered de-ionized water (Li et al. 2001b) at temperatures 1°C and 21°C respectively. For the low pH

waters on the other hand, the synergistic effect was found to be higher, compared to the high pH waters. The $Ct_{1\text{-log}}$ values were found to be considerably lower and they were comparable to those of earlier studies with buffered de-ionized water (Table 4.13). The results were thus consistent with the hypothesis that high pH and alkalinity limited the synergistic effect in natural waters A to E.

Water pH was an important factor influencing effectiveness of chlorine as a disinfectant. A general hypothesis for the mechanism of the synergistic effect was that the primary treatment with ozone increases the permeability of the oocyst wall by oxidative attack. The HOCl molecule can then more readily penetrate into the interior of the oocyst to inactivate the more vulnerable sporozoites (Li et al. 2001b). Hypochlorous acid, HOCl, which is the predominant form of free chlorine at pH 6 is generally considered to be a more effective biocidal agent than hypochlorite ion, OCl^-, the predominant form at pH 8. In de-ionized water, 96% of the free chlorine exists in the HOCl form at pH 6.0, whereas at pH 8.0 only 26% exists in the HOCl form (Haas 1999). The DPD assay used to measure free chlorine in this study, however, does not distinguish between HOCl and OCl^- species or relative biocidal effectiveness at different pH. The high pH natural waters were naturally buffered by carbonate/bicarbonate alkalinity. In order to investigate the effect of a reduction to pH of 6 on the synergistic effect, the pH of the high pH natural waters was adjusted to 6 in some of the trials by adding acid. However, during the experiment, the pH was observed to slowly drift upward during the period of exposure to the secondary oxidant. Li et al (2001b) on the other hand conducted their experiments in de-ionized water that was well buffered at a pH of 6.0. Thus it may be hypothesized that the synergistic effect of ozone followed by free chlorine in the high pH natural waters was inhibited mainly as a result of a pH effect.

In general, pH change in natural waters is a complex phenomenon, governed by several factors. Natural waters acquire their chemical characteristics by dissolution and by chemical reactions with solids, liquids, and gases with which they have come into contact. Waters vary in their chemical composition, but these variations are at least partially understandable if the environmental history of the water and the chemical

reactions of the rock-water-atmosphere systems are considered (Stumm and Morgan, 1996).

The pH of most natural waters is controlled by reactions involving the carbonate system. In natural waters containing significant amount of $CaCO_3$ (calcite) like the ones used in this study, the amount of $CaCO_3$ dissolved depends on the initial CO_2 concentration and on the extent to which the CO_2 in the water can be replenished by exchanging with a gas phase. If CO_2 is not replenished (the system is closed to exchange of CO_2 gas), the amount of calcite that natural water can dissolve is essentially limited by the amount of CO_2 present initially, since dissolution follows the equation

$$CaCO_3 + H_2O + CO_2 \Leftrightarrow Ca^{+2} + 2\,HCO^{-1} \qquad \text{Equation 4.3}$$

If the system is open to CO_2, the CO_2 from the gas phase will be transferred to replace the CO_2 consumed by dissolution of calcite. In this case, the dissolution of calcite is not limited by the availability of CO_2. More calcite will dissolve under open-system conditions than under closed-system conditions. In the present study, the raw waters were stored at 4°C in closed vessels, and hence were under "closed-system condition". Before the experiments, the waters were taken out of the vessels and were kept under "open-system condition". In the past, differences have been observed between field and laboratory determinations of the pH values that are ascribed to the carbonate system. Such differences were observed by Roberson et al. (1963), who examined the ground and surface waters of Sierra Nevada, California (Faust and Aly, 1981). The time interval between the field and laboratory determinations ranged from 5 to 120 days, which was similar to the period used for this study. In their study there were some indications that laboratory determinations made within one week showed somewhat smaller changes than samples stored for longer periods of time. There seemed to be a tendency for waters with a "low" total carbonate content to gain CO_2 and to show a decrease in pH value, whereas waters with a "high" total carbonate content tended to lose $CO_2(g)$ to the atmosphere and to show an increase in pH value. In the present study most of the waters had high total

carbonate content. When transferred from a closed system to an open sytem, the waters might have lost $CO_2(g)$ to the atmosphere and hence showed the increase in the pH value.

In other cases it has been found earlier (Stumm and Morgan, 1996) that when a large quantity of acid is discharged into a natural water system containing $CaCO_3$ solids, an initially large decrease in the pH of that system occurs. The final pH change is much less than the initial decrease. This is mainly attributed to the buffering capacity of many natural waters. The decrease in pH resulting from the addition of the acid leads to the dissolution of solid calcium carbonate and the establishment of a new equilibrium position. To test this explanation for the pH change phenomena observed during this study, samples of the high pH natural waters were filtered through 0.22 µm filters prior to pH adjustment. Despite the filtration step, a similar increase in pH was observed during the free chlorine contact time. This indicated that pH of the studied natural waters was not controlled entirely by reactions involving the carbonate system. The experience in the laboratory during this study therefore suggests that, in water treatment practice, it may be difficult to promote a synergistic effect on *C. parvum* in alkaline waters by filtration followed by pH reduction using a mineral acid.

In natural waters, often, more significant than the buffer contribution of dissolved carbonic species are the many heterogeneous chemical, biochemical, and physical processes that occur in waters in their natural surroundings. Dissolution and deposition of minerals, ion exchange equilibria between soluble components and silicate minerals or clays, photosynthesis and biologic respiration, and aeration are typical processes affecting buffer action in natural water systems (Weber and Stumm, 1963). Other biologically mediated reactions like the ones mentioned in Table 4.14, also affect the pH of the natural waters. Thus, the pH of natual water is determined essentially by the interactions of biological activities and heterogeneous and homogeneous equilibria. The dissolved-carbonate system, then, although an important mediator and indicator of the buffer capacity of natural waters, represents only a fraction of the total capacity rather than the sole or principle buffering agent (Weber and Stumm, 1963).

*Table 4.14: Biologically mediated reactions affecting pH in natural water systems

Process	Reaction	Effect on pH
Photosynthesis	$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$	Increase
Respiration	$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$	Decrease
Methane Fermentation	$C_6H_{12}O_6 + 3CO_2 \rightarrow 3CH_4 + 6CO_2$	Decrease
Nitrification	$NH^{4+} + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$	Decrease
Denitrification	$5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30CO_2 + 12N_2 + 42H_2O$	Increase
Sulfide Oxidation	$HS^- + 2O_2 \rightarrow SO_4^{-2} + H^+$	Decrease
Sulfate Reduction	$C_6H_{12}O_6 + 3SO_4^{-2} + 3H^+ \rightarrow 6CO_2 + 3HS^- + 6H_2O$	Increase

*Table obtained from Weber and Stumm, 1963.

4.5.2 Effect of Water Quality

According to the regression analysis in Section 4.2.3, the effect of water quality was found to be insignificant on the synergistic effect in the high pH natural waters. However, it is possible that due to the inhibition of the synergistic effect (Table 4.13) in the high pH natural waters, the effect of water quality may have been diminished. With the low pH natural waters, the $Ct_{1\text{-log}}$ values (Table 4.13) were comparable to that of the earlier studies with buffered de-ionized water. The $Ct_{1\text{-log}}$ values for waters F and G though close, were about two times greater than that of buffered de-ionized water under identical conditions. Natural waters F and G were similar in terms of the measured water quality parameters, such as total organic carbon, colour, and turbidity (Table 3.3). Since the conditions were identical (including pH) for both the waters F and G and that of the buffered de-ionized water, this may be an indication of a water quality effect. The dataset

obtained with natural waters F and G in part III is limited and this makes it difficult to draw firm conclusions regarding the influence of water quality characteristics on the synergistic effect. However, they do suggest a possible role of other water quality characteristics in determining the synergistic effect in natural waters F and G.

4.5.3 Effect of Ozone Pre-treatment and Temperature

The regression analysis done with high pH natural waters demonstrated that the effect of ozone pretreatment level and temperature was found to be insignificant on the synergistic effect. In contrast to the results of this study with high pH natural waters, both the level of ozone pre-treatment and temperature had significant effects on the magnitude of the synergistic effect in earlier studies with buffered de-ionized water (Li et al. 2001b). By definition, ozone pre-treatment must be an important variable at some level if a synergistic effect exists. The magnitude of the synergistic effect measured in this study with high pH waters, however, was relatively small compared to the experimental variability. Due to this inhibition of the synergistic effect in high pH natural waters, the effect of ozone pre-treatment level and temperature may have been inhibited. The effects of ozone pre-treatment level and temperature, therefore, may simply have been too small to detect with statistical significance against the background variability.

In the two sets of experiments with natural water F, the synergistic effect measured at 21°C was more than 5 times that measured at 5°C for comparable ozone primary treatment levels. This temperature dependence was similar to that reported by Li et al. (2001b) indicating that temperature probably played a role in determination of the synergistic effects in natural waters, much like as it has been reported to do in buffered de-ionized water. The effect of ozone pretreatment also may have been significant in the low pH waters. However, in this study sufficient data were not available to make such a conclusion. The dataset obtained with natural waters F and G in part III was limited and this makes it difficult to draw firm conclusions regarding the influence of ozone primary treatment level on the synergistic effect.

4.5.4 Comparison with Other Studies

A comparison of this study with the study by Li et al. (2001b) has already been discussed. There were also some other studies conducted earlier by different research groups (Oppenheimer et al. 2000, Driedger et al. 2000, Rennecker et al. 2000, Corona-Vasquez et al. 2002), which investigated the synergistic effect of ozone followed by free chlorine sequential treatment of *C. parvum*.

Studies conducted by Oppenheimer et al. (2000) in different natural waters found the synergistic effect to be inconsistent in the various waters tested. They cited the limited precision of the animal infectivity assay and a poor understanding of the factors responsible for the synergistic effect in the natural water environment.

Other research groups conducted their experiments in buffered de-ionized water and used in-vitro excystation as a method of viability assessment. Driedger et al. (2000) found a high influence of pH on the synergistic inactivation of *C. parvum*. The greatest level of synergy was observed at pH 6; synergy decreased as pH increased until no synergy was observed at pH 8.5. This is consistent with the findings of this study, indicating that hypochlorous acid is the main component of the free chlorine species, which was responsible for the *C. parvum* inactivation. Contrary to the findings of this study, Rennecker et al. (2000a) concluded a high dependence of synergistic effect on temperature. A stronger synergy was observed at a lower temperature. The rate of free chlorine inactivation was 1.1 and 2.8 times faster than the corresponding rate of ozone primary inactivation at the respective temperatures of 30 and 10°C.

CHAPTER 5: SEQUENTIAL INACTIVATION OF *C. PARVUM* WITH OZONE FOLLOWED BY MONOCHLORAMINE IN NATURAL WATERS

5.1 INTRODUCTION

The purpose of the experiments described in this chapter was to provide a detailed evaluation of the synergistic effect during the inactivation of *C. parvum* oocysts in natural waters for ozone followed by monochloramine sequential treatment. The specific research objectives were to determine which of the following experimental factors have a significant impact on the synergistic effect for ozone followed by monochloramine sequential treatment: (1) level of primary ozone treatment; (2) pH of the water; (3) temperature of the water and (4) quality of the water.

5.2 EXPERIMENTAL DESIGN

The approach adopted for the determination of the synergistic effect was similar to that of the earlier experiments with ozone followed by free chlorine sequential treatment. A 2^{4-1} factorial designed experiment was used to investigate the synergistic effect in two natural waters. The design matrix and the levels of the experimental factors are described in Table 5.1. The 2^{4-1} fractional factorial experiment permits determination of the main effects of four experimental factors (or variables) with only eight experimental settings and ensures that the main effects are free from confounding with second-order interaction effects (Box et al. 1978). Triplicate trials at each experimental condition were conducted for ozone, and monochloramine, as well as the control trials.

Table 5.1: The 2^{4-1} design matrix for ozone followed by monochloramine sequential treatment

Factors		Experimental Factor Targets	
		-1	1
1	Natural Water	D	E
2	Ozone Pre-treatment Level	Low (0.7 log-unit)	High (1.7 log-unit)
3	pH	Initial pH 6.0	8.1
4	Temperature (°C)	1	21

Trial No.	[a]Factor Levels in Scaled-Units				No. of Replicates
	1	2	3	[b]4=123	
1	1	1	1	1	3
2	-1	1	1	-1	3
3	1	-1	1	-1	3
4	-1	-1	1	1	3
5	1	1	-1	-1	3
6	-1	1	-1	1	3
7	1	-1	-1	1	3
8	-1	-1	-1	-1	3

[a]Identification of the factors is provided at the top of the table
[b]Dummy factor used to generate the fractional factorial design.

5.3 RESULTS

Results of sequential treatment trials with the two natural water samples are summarized in Figures 5.1 to 5.4. Details of the infectivity reduction for these trials are provided in Tables B.4 and B.5 of Appendix B. Details of the ozone primary treatment conditions are provided in Tables C.4 and C.5 of Appendix C. Details of the secondary treatment conditions with monochloramine are provided in Tables D.4 and D.5 of Appendix D. In figures 5.1 to 5.4, the total *C. parvum* inactivations (measured after different levels of exposure to the monochloramine, both with and without ozone pre-treatment) versus the product of the average monochloramine concentration and the exposure time, *Ct*, are plotted. The data points located at the x-axis origin, where monochloramine $Ct = 0$, are a direct measure of the inactivations following exposure to ozone but prior to addition of monochloramine. At 21°C, the experimental ozone *Ct* products were between 0.6 and 2.0 mg·min/L for the 0.7 log-unit ozone pre-treatment target and between 2.5 and 4.0 mg·min/L for the 1.7 log-unit pre-treatment target. Larger ozone *Ct* products were required to achieve similar inactivation targets at the lower experimental temperatures due to the temperature dependence of *C. parvum* inactivation by ozone (Li et al. 2001). At the lower temperature, the experimental ozone *Ct* products were between 6.0 and 9.5 mg·min/L for the 0.7 log-unit ozone pre-treatment target and between 10.0 and 27.0 mg·min/L for the 1.7 log-unit pre-treatment target. The objective was to maintain similar primary inactivation levels at the two temperatures, rather than similar *Ct* exposure levels.

For some of the low temperature trials, the target temperature of 1°C, was not achieved due to difficulties with the cooling unit, and the actual experimental temperature was 5°C (Figure 5.2). In the low pH experiments, the pH of the water samples was lowered prior to experiments from 8.1 to 6.0 prior to experiments by acid addition. Despite the overnight stabilization period, with the reactor contents constantly stirred and open to atmosphere, the pH was observed to slowly increase from the initial pH of 6.0 during the 4 hours monochloramine exposure period. These experiments were, therefore, identified as "initial pH of 6.0" and were not conducted at a truly stable pH of 6.0.

In a few cases, the infectivity results were above the upper detection limit of the neonatal CD-1 mouse assay. That is, none of the mice in the group that received the largest inoculum (typically 100 000 oocysts) became infected. These points are indicated by the " > " sign in the figure legends. For the purpose of computing the k, the infectivity reductions for these data were set at the detection limit (i.e. by assuming one mouse in the cohort was infected). Using this approach, the computed k values were biased toward a conservative interpretation of the effectiveness of the given treatment. Often these data were found to lie close to other data points for which the infectivity result was within the detection limit.

The solid and dashed lines in Figures 5.1 to 5.4 represent the least-squares best-fit of the first-order Chick-Watson rate equation (Equation 3.7) to the experimental data. A synergistic effect was evident if the rate of inactivation by monochloramine was greater with ozone pre-treatment exposure than without. Based on this criterion, there was evidence of a synergistic effect with both natural water samples and for most of the treatment variable combinations investigated.

The calculated Chick-Watson rate constants, (k'), for each of the trials shown in Figures 5.1 to 5.4 are provided in the Tables 5.2 and 5.3. The 90% confidence intervals on the values of k, are also reported in Tables 5.2 and 5.3. If the computed 90% confidence interval on the value of k did not include zero, the computed k was determined to be statistically different from zero. If the computed k value for monochloramine inactivation (k_2) with ozone primary treatment was greater than the computed k value for monochloramine inactivation without primary treatment (k_1), then this was considered as evidence of a synergistic effect (Figure 3.1). The difference between the slopes (k_2-k_1) was considered to be statistically significant when the level of significance (p-values) of the test statistic (Section 3.6.2 in Chapter 3) was less than 0.1. The last column in each of the tables (5.2 and 5.3) indicates if the synergistic effect measured with ozone primary treatment was statistically significant at the 90 % confidence level (p<0.1). Based on

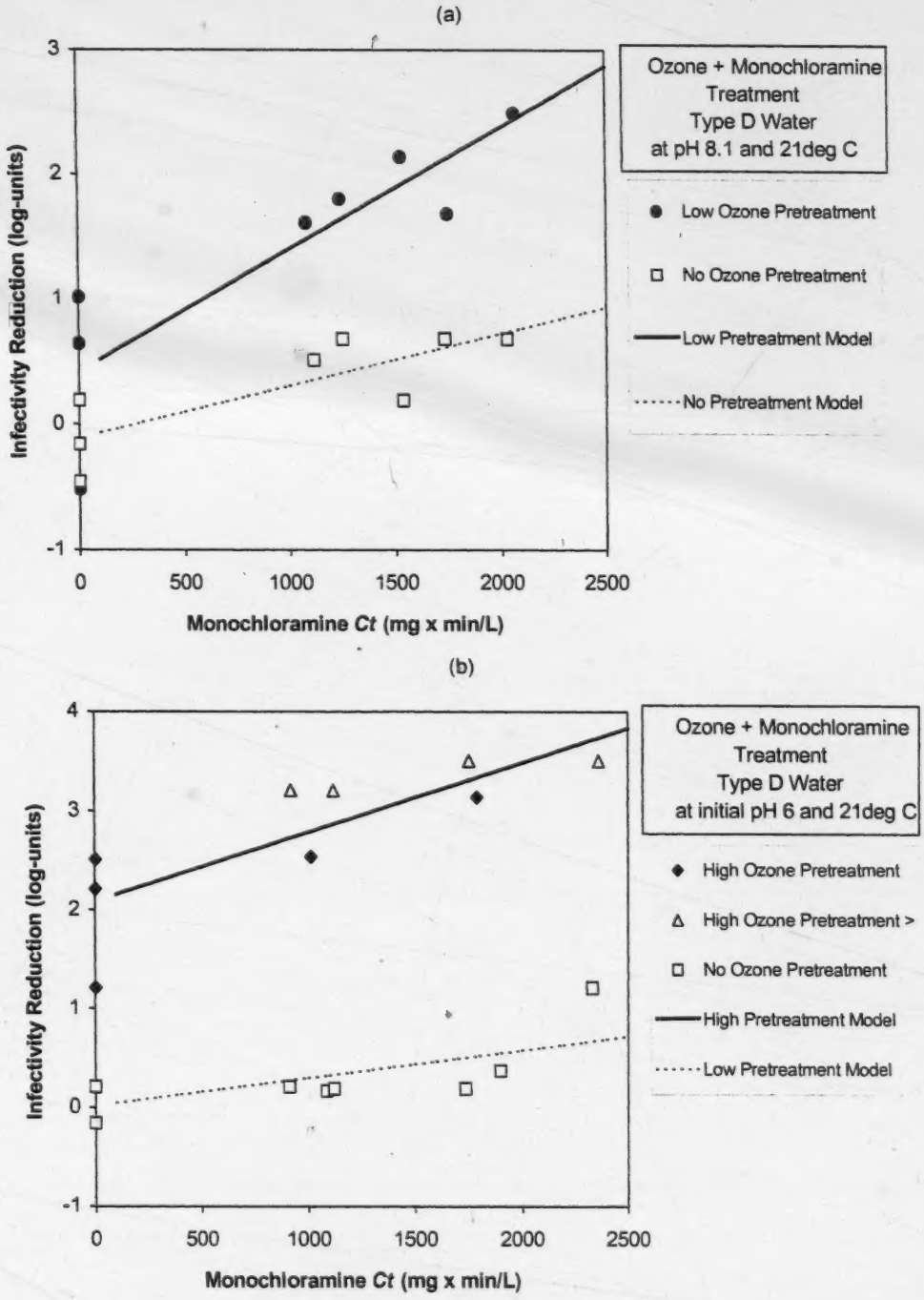

Figure 5.1 Effect of ozone and monochloramine treatment on *C. parvum* oocysts in natural water D at (a) pH 8.1 and (b) initial pH 6 at 21 °C.

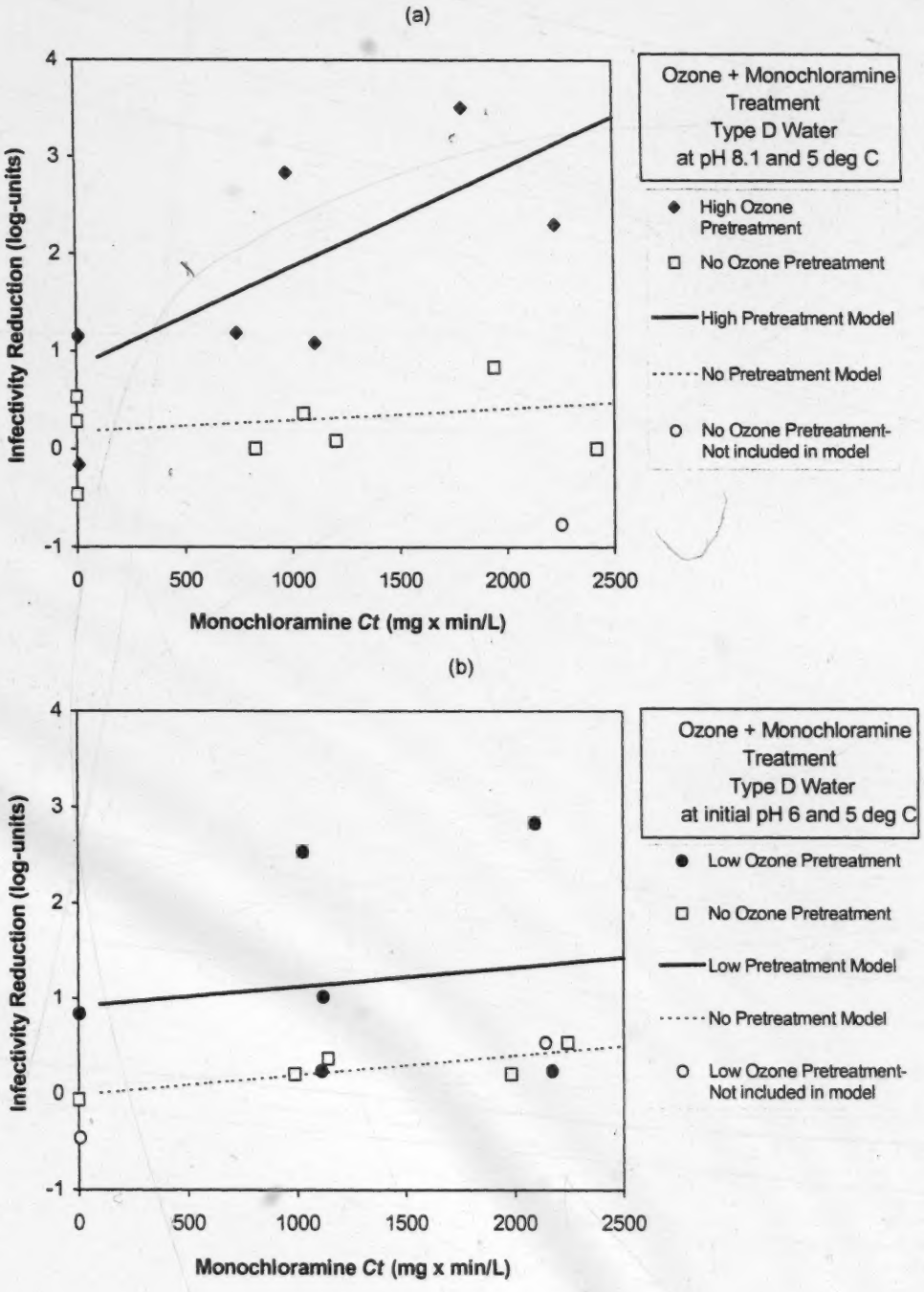

Figure 5.2 Effect of ozone and monochloramine treatment on *C. parvum* oocysts in natural water D at (a) pH 8.1 and (b) initial pH 6 at 5 °C.

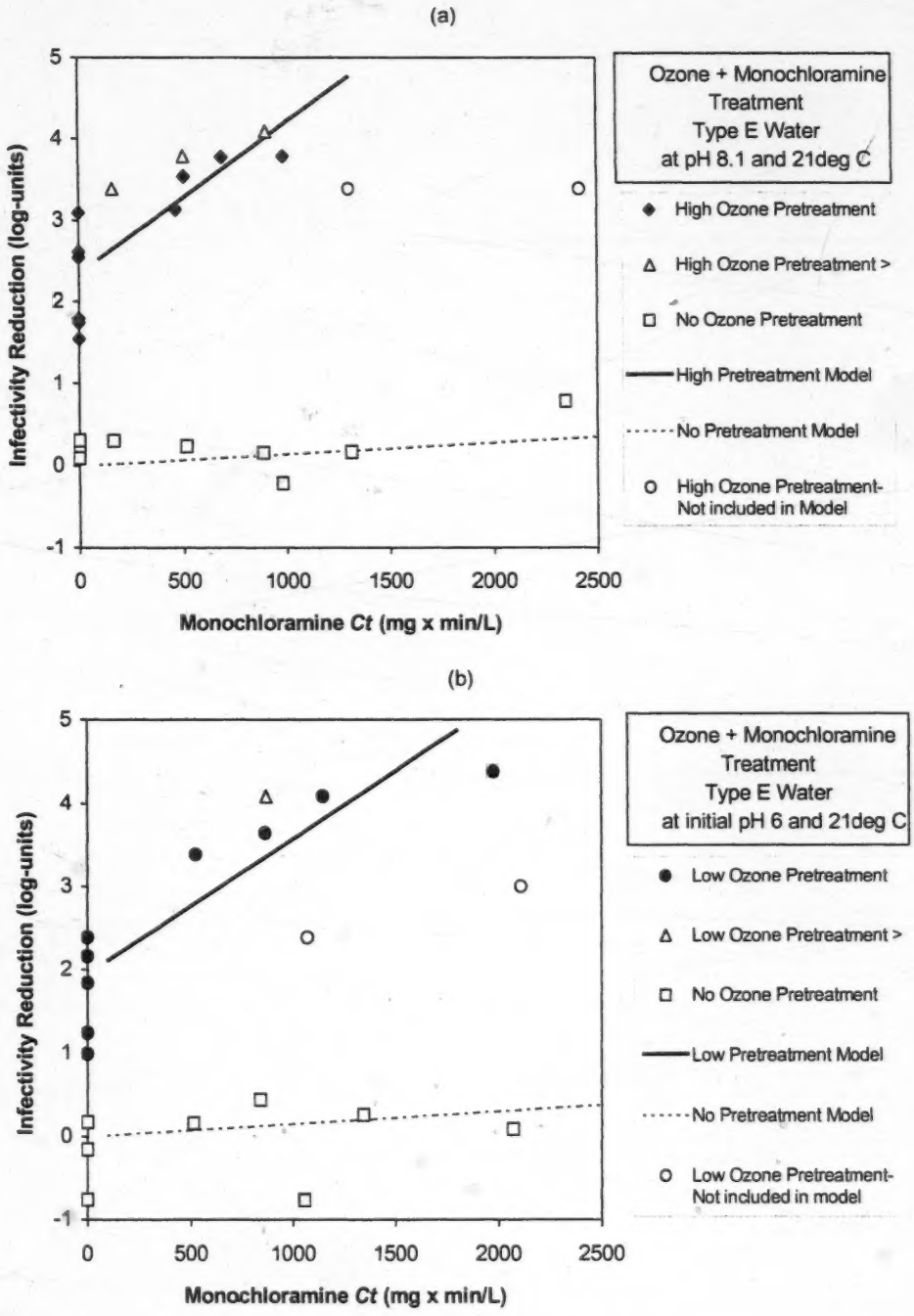

Figure 5.3 Effect of ozone and monochloramine treatment on *C. parvum* oocysts in natural water E at (a) pH 8.1 and (b) initial pH 6 at 21 °C.

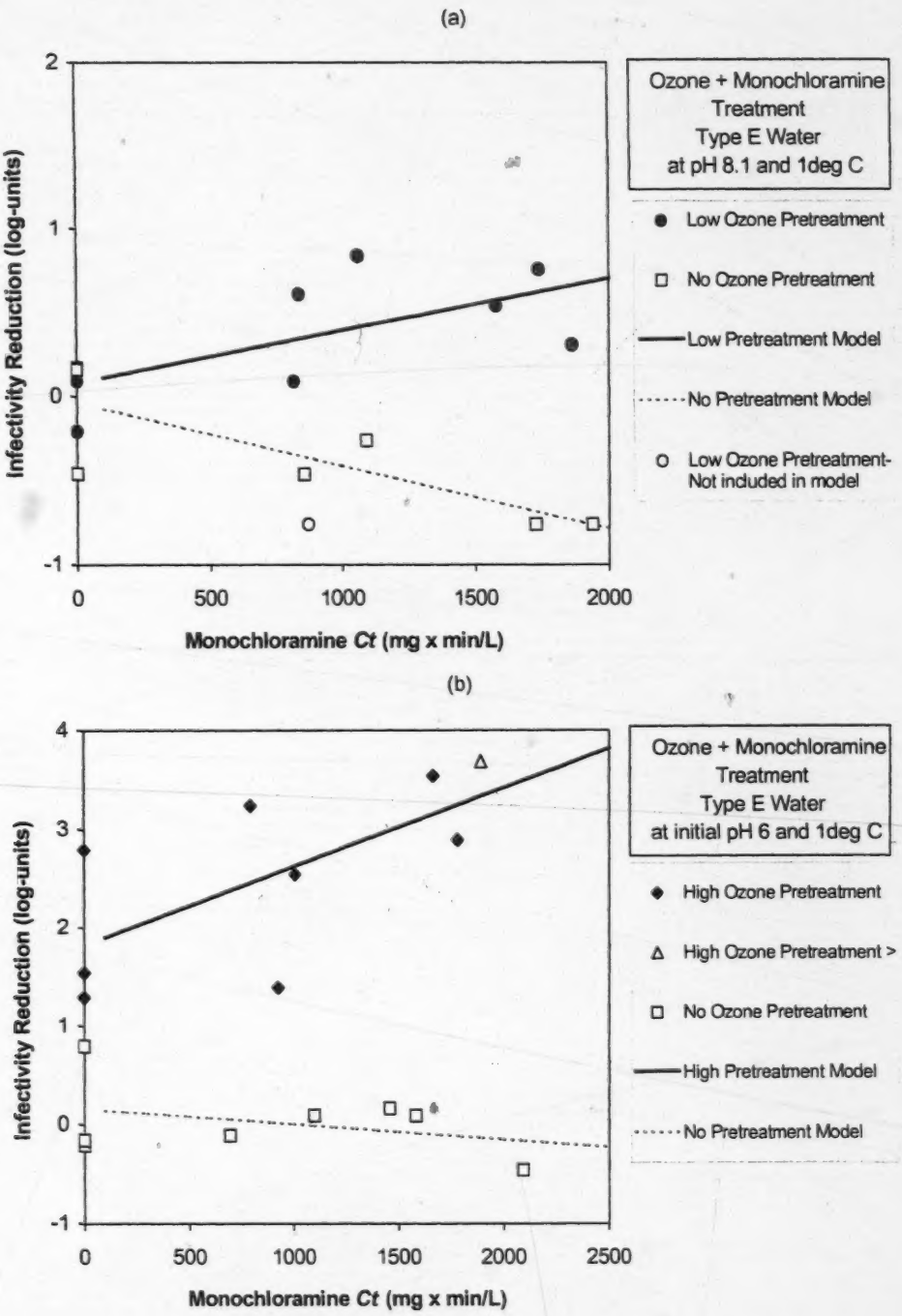

Figure 5.4 Effect of ozone and monochloramine treatment on *C. parvum* oocysts in natural water E at (a) pH 8.1 and (b) initial pH 6 at 1 °C.

Table 5.2: Calculated Chick-Watson rate constants for ozone followed by monochloramine sequential trials with natural water D

Fig. No.	pH	Temp. (°C)	Ozone Primary Treatment Level (log-units)	$k \pm 90\ \%$ CI[a] ($\times 10^4$ L/mg/min)	[b]Significant k?	$(k_2\text{-}k_1) \pm 90\ \%$ CI[a] ($\times 10^4$ L/mg/min)	[c]Significant Synergistic Effect? (p-values)
5.1 (a)	8.1	21	0.42	9.8 (7.0, 12.6)	Yes	5.7 (3.6, 7.7)	Yes (0.0004)
	8.1	21	0	4.2 (2.7, 5.6)	Yes		
5.1 (b)	[d]6.0	21	2.1	7.0 (4.7, 9.3)	Yes	4.2 (2.5, 5.9)	Yes (0.0008)
	[d]6.0	21	0	2.8 (1.5, 4.1)	Yes		
5.2 (a)	8.1	5	0.84	10.0 (5.4, 14.6)	Yes	9.1 (5.9, 12.4)	Yes (0.0003)
	8.1	5	0	1.2 (-1.1, 3.5)	No		
5.2 (b)	[d]6.0	5	0.91	2.0 (-6.2, 10.2)	No	0.002 (-5.7, 5.7)	No (0.99)
	[d]6.0	5	0	2.0 (0.99, 3.1)	Yes		

[a]Confidence Interval
[b]Considered significant if 90% confidence interval on the value of k do not include zero
[c]Considered significant if the p-value of the test statistic is less than or equal to 0.1
[d]Initial pH 6.0

Table 5.3: Calculated Chick-Watson rate constants for ozone followed by monochloramine sequential trials with natural water E

Fig. No.	pH	Temp. (°C)	Ozone Primary Treatment Level (log-units)	$k \pm 90\%$ CI[a] ($\times 10^4$ L/mg/min)	[b]Significant k ?	$(k_2-k_1) \pm 90\%$ CI[a] ($\times 10^4$ L/mg/min)	[c]Significant Synergistic Effect? (p-values)
5.3 (a)	8.1	21	2.3	18.6 (12.8, 24)	Yes	17.2 (14.3, 20.1)	Yes (4.0×10^{-9})
	8.1	21	0	1.4 (0.06, 1.6)	Yes		
5.3 (b)	[d]6.0	21	1.95	16.3 (11.8, 21)	Yes	14.7 (11.0, 18.4)	Yes (6.0×10^{-6})
	[d]6.0	21	0	1.5 (-1.8, 4.8)	No		
5.4 (a)	8.1	1	0.081	3.1 (1.5, 4.7)	Yes	6.9 (5.4, 8.4)	Yes (3.0×10^{-6})
	8.1	1	0	-3.7 (-5.5, -2.1)	No		
5.4 (b)	[d]6.0	1	1.8	8.0 (4.0, 12.0)	Yes	9.6 (6.4, 12.7)	Yes (0.00012)
	[d]6.0	1	0	-1.5 (-3.7, 0.7)	No		

[a]Confidence Interval
[b]Considered significant if 90% confidence interval on the value of k do not include zero
[c]Considered significant if the p-value of the test statistic is less than or equal to 0.1
[d]Initial pH 6.0

Table 5.4: Estimated monochloramine *Ct* products required for a 1 log-unit synergistic effect for ozone followed by monochloramine sequential treatment in the designed experiments

Natural Water	Water Conditions	Ozone Primary Treatment Level (log-units)	*Ct* Required for 1 log-unit Synergistic Effect (mg×min/L)	Statistically significant?
D	21°C, pH 8.1	0.42	1 750	Yes
D	21°C, pH 6.0[a]	2.1	2 300	Yes
D	5°C, pH 6.0[a]	0.91	5 000 000	No
D	5°C, pH 8.1	0.84	1 100	Yes
E	21°C, pH 8.1	2.3	580	Yes
E	21°C, pH 6.0[a]	1.95	680	Yes
E	1°C, pH 6.0[a]	1.8	1 040	Yes
E	1°C, pH 8.1	0.081	1 450	Yes

[a]Initial pH 6.0

these tables a statistically significant synergistic effect was observed in almost all the experimental conditions investigated with both waters D and E. The exception was for water D at low ozone primary treatment at initial pH 6 and temperature of 5°C. To help compare the magnitudes of the synergistic effect measured in the different waters and at the various treatment conditions, *Ct* products required for a 1 log-unit synergistic effect were estimated from Tables 5.2 and 5.3, and then summarized in Table 5.4. The predictive equation 4.1 was used to generate the estimates of synergistic effect. A significant synergistic effect was observed for the majority of the ozone and monochloramine sequential trials (Table 5.4). The information in Table 5.4 however, shows a wide range in the monochloramine *Ct* products required for a synergistic effect and suggests that there was considerable variability in the synergistic effect.

5.4 CONTROL TRIALS

Inactivation of oocysts from the 42 positive control reactors used in this study ranged from –0.8 to 0.8 log-unit with a mean of 0.12 log-unit. This range in oocyst inactivation in unexposed control samples is typical and reflects the normal variation in the neonatal CD-1 mouse assay. In comparison, the range of inactivation measured in the positive controls in a previous *C. parvum* sequential inactivation study carried out in phosphate buffered de-ionized water (Li et. al 2001b) ranged from –0.8 to 0.9 log-unit, with a mean of 0.03 log-unit inactivation. Histograms showing the reduction in infectivity of the oocysts in the control reactors for each natural water, are presented in Appendix E. The mean inactivation in the positive controls indicated that the exposure to the experimental water matrices and the various processing steps had little effect on mean oocyst infectivity. The control trials served primarily as a quality assurance for the experiments.

5.5 STATISTICAL ANALYSIS

The results of the factorial designed experiment with ozone followed by monochloramine sequential treatment are summarized in Table 5.5. The results

summarized in Table 5.5 were analyzed using multiple linear regression to determine which experimental factors had a significant impact on the synergistic effect. To facilitate the multiple linear regression analysis, the levels of the experimental factors in Table 5.5 was first scaled so that the levels of each ranged from approximately –1 to +1. The scaling is described in Table 5.6. Using the scaled values of the variables a multi-linear model of the form described in equation 3.9 was used for linear regression. The dependent variable in equation 3.9, Y represented the magnitude of the synergistic effect, k_2-k_1, and the independent variables X_1, X_2, X_3, and X_4 represented ozone primary treatment level, temperature, pH, and water quality, respectively. Regression analysis was done using the regression tool in Microsoft Excel 2000. Model terms were rejected or retained based on the statistical significance of the coefficients a_0, a_1 …etc. at the 90% confidence level as described previously (Draper and Smith, 1966). Based on this approach, the variables X_1, X_3, and X_4 were found to be statistically significant at the 90%

Table 5.5: Summary of synergistic effect determinations for the factorial design experiments with ozone followed by monochloramine sequential treatment

Result No.	Natural Water	*Ozone Pre-treatment (Inactivation log-units)	Temperature (°C)	pH	Synergistic Effect (k_2-k_1) [$\times 10^4$ L/mg/min]
1	D	0.42	21	8.1	5.7
2	D	2.1	21	Initial 6.0	4.2
3	D	0.91	5	Initial 6.0	0.002
4	D	0.84	5	8.1	9.1
5	E	2.3	21	8.1	17.2
6	E	1.95	21	Initial 6.0	14.7
7	E	1.8	1	Initial 6.0	9.6
8	E	0.081	1	8.1	6.9

*The values shown represent an average of the triplicates

Table 5.6: Results from the factorial design experiment of the sequential trials using ozone and monochloramine sequential treatment represented in terms of scaled variables

Factors	-1	1
Ozone Primary Treatment Level (log units)	0.7	1.7
Temperature (°C)	1	21
pH	Initial pH 6.0	8.1
Natural Water	D	E

Result No.	Synergistic Effect (Y)	Scaled Experimental Variables			
		Ozone Primary Treatment (X_1)	Temp. (X_2)	pH (X_3)	Water Quality (X_4)
1	5.7	-1.56	1	1	-1
2	4.2	1.8	1	-1	-1
3	0.002	-0.58	-0.6	-1	-1
4	9.1	-0.72	-0.6	1	-1
5	17.2	2.2	1	1	1
6	14.7	1.5	1	-1	1
7	9.6	1.2	-1	-1	1
8	6.9	-2.2	-1	1	1

confidence level and equation 3.9 reduced to the form:

$$Y = 8.0 + 2.2X_1 + 3.0X_3 + 2.6X_4 \qquad \text{Equation 5.1}$$

The variable temperature was not found to be statistically significant and was eliminated from the regression. Water quality, pH and ozone pre-treatment level were all found to be significant equation variables at the 90% confidence level (i.e. $p < 0.10$) and were retained. After scaling back to the original form the final regression equation describing the synergistic effect observed under the conditions investigated was:

$$(k_2 - k_1) = -17.6 + 2.6 \times (\text{Water Quality A/B}) + 2.9 \times (\text{pH}) + 4.4 \times (O_3 \text{ Pre} - \text{Treatment})$$

Equation 5.2

The overall model and the individual parameters in it were significant at the 90% level. Values of the regression equation coefficients and associated confidence intervals and *p*-values are provided in Table 5.7. The fit of the regression model is shown in Figure 5.5 and the model predictions matched the observations satisfactorily.

Table 5.7: ANOVA analysis results of the sequential trials with natural waters D and E using ozone followed by monochloramine

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.92
R Square	0.85
Adjusted R Square	0.74
Standard Error	2.84
Observations	8

ANOVA

	df	SS	MS	F	Significance F
Regression	3	186.40	62.13	7.69	0.039
Residual	4	32.30	8.07		
Total	7	218.7			

	Coefficients	Standard Error	t Stat	P-value	Lower 90.0%	Upper 90.0%
Intercept	-17.63	9.18	-1.92	0.13	-37.2	1.9
Ozone Kill	4.46	1.57	2.84	0.05	1.1	7.8
pH	2.87	1.12	2.56	0.06	0.5	5.3
Water Type	2.64	1.07	2.47	0.07	0.4	4.9

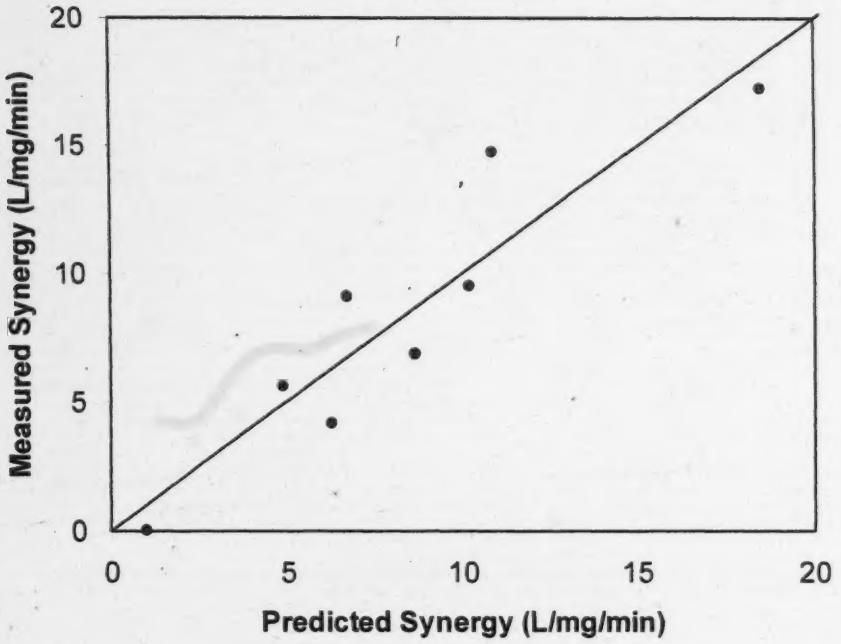

Figure 5.5 Fit of the linear regression model for predicting the synergistic effect of ozone followed by monochloramine sequential treatment for the inactivation of *C. parvum* oocysts in natural waters D and E. (Straight line represents perfect fit).

5.6 DESIGN CRITERIA

Based on the model developed for determining the level of synergistic inactivation of *C. parvum* in natural waters, design-criteria were developed (Table 5.8) for a specified level of inactivation at a set of conditions such as level of ozone pre-treatment, pH, and water type. Since only two types of waters were used for the study, the design criteria are only applicable for those waters of similar characteristics to the waters D and E. Extrapolation of these results should not be done as the *Ct* requirements may change dramatically under different conditions. The design criteria shown in Table 5.8 are only applicable at a pH near 8.1 and not for any other pH higher or lower than 8.1. The developed model (equation 5.2) indicated that at lower pH, the synergistic effect was lower compared to the higher pH. However, no design criteria were developed at the lower pH since the pH was not stable under those conditions and hence the pH effect might have been underestimated. Since experiments were not done beyond 2 log-units ozone pretreatment, the *Ct* requirements under such conditions were not included in Table 5.8.

Table 5.8 Monochloramine *Ct* requirements for the synergistic inactivation of *C. parvum* due to ozone followed by monochloramine sequential treatment in natural waters D and E at pH 8.1

Synergistic effect in terms of infectivity reduction (log-units)	Monochloramine *Ct* requirements (mg×min/L)			
	1 log infectivity reduction by ozone pre-treatment		2 log infectivity reduction by ozone pre-treatment	
	Water D	Water E	Water D	Water E
1.0	940	630	780	555
2.0	1 885	1 265	1 560	1 110
3.0	2 830	1 900	2 340	1 670

Table 5.9 Comparison of model predictions of *Ct* required for 1 log-unit synergistic effect between laboratory de-ionized water and natural waters for ozone followed by monochloramine sequential treatment at pH 8

Temp. (°C)	Ozone Pre-treatment (log-units)	[a]$Ct_{1\text{-log}}$ (mg×min/L)		
		[b]Buffered Water	[c]Natural Water D	[c]Natural Water E
22	1.6	590	1 000	660
22	0.4	990	2 050	990
1	1.6	1 810	1 000	660
1	0.4	3 790	2 050	990

[a]*Ct* required for 1 log-unit synergistic effect
[b]Calculated using model predictions from Li et al. (2001b) and Equation 4.1
[c]Calculated using Equations 4.1 and 5.2

5.7 DISCUSSIONS

To help compare the magnitudes of the synergistic effect measured in the different waters and at the various treatment conditions, the monochloramine *Ct* products required for 1 log-unit of synergistic effect are summarized in Table 5.9 using the results from this study with natural waters D and E and the results from a previous study (Li et al. 2001b) with buffered de-ionized water. For the natural waters, the predictive equations 4.1 and 5.2 were used to generate the estimates of synergistic effect.

From Table 5.9, it was found that the magnitudes of the synergistic effect predicted for both the natural waters were comparable to those for buffered de-ionized

water. At 22°C, there was no significant difference between the synergistic effects in buffered de-ionized water and the natural waters for both high and low ozone pre-treatment. There were some differences in synergistic effect at low temperatures for both low and high ozone pretreatment. However, overall, the data suggest that the synergistic effect of ozone followed by monochloramine sequential treatment that was measured previously in buffered de-ionized water was not inhibited in natural waters.

5.7.1 Effect of Water Quality

From Table 5.9, it was found that the monochloramine *Ct* product required to produce the equivalent synergistic effect in natural water D was approximately twice that required for natural water E or for de-ionized water. Of the two natural waters studied, natural water E was considered to be of higher quality because it was lower in turbidity, colour and total organic carbon than natural water D (Table 3.3). These results imply that for relatively good quality natural waters, the synergistic effect of ozone and monochloramine sequential treatment is comparable to that determined in de-ionized water. As the water quality deteriorates, the concentration of impurities and constituents in natural water increase to a level where they may interfere with the mechanism that accounts for the synergistic effect. While insufficient to conclude, the data presented here suggest that the presence of suspended solids, colloidal material or dissolved organic material in natural waters may inhibit the synergistic effect. These constituents are related to the water quality measurements of turbidity, colour and total organic carbon. It is equally possible that other, unidentified, constituents present at higher concentration in natural water D were responsible for the reduced synergistic effect in that water.

Determining the reasons for the influence of water quality characteristics on the synergistic effect calls for an investigation of the mechanism of inactivation at the molecular level. Unfortunately this was beyond the scope of this study. There are also very few studies in the past, which actually investigated the mechanism of inactivation using two or more oxidants sequentially. A general hypothesis for the mechanism of synergy is that the strong oxidants in the primary treatment increase the permeability of

the oocyst wall by physically damaging or altering its surface properties. Without pre-treatment, diffusion of both free chlorine and monochloramine (chlorine species) through the oocyst wall is the rate-limiting step in the diffusion-reaction process. Since ozone is a very strong oxidant, and it can oxidize many organic materials including lipid and proteins, it was postulated earlier (Li et al. 2001b) that the ozone pre-treatment increased oocyst wall permeability by weakening the oocyst wall. For natural waters, a similar mechanism will probably be true. The parameters turbidity, colour, and TOC of the natural waters may interfere with the rate-limiting step of the diffusion-reaction process of the chlorine species. These parameters may introduce an additional barrier in the diffusion process, which may reduce the level of oxidant penetration in the oocyst and hence result in a reduced synergistic effect. The sequential addition of ozone and chlorine species in natural waters with higher turbidity, colour, and TOC may also result in reactions with dissolved, colloidal, and particulate matter in the natural waters. These reactions might interfere with some of the reactions responsible for the oocyst inactivation and hence show a lower synergistic effect. However, all these explanations are only speculative and can only be confirmed by additional research.

5.7.2 Effect of pH

It may be hypothesized that the synergistic effect of ozone followed by monochloramine in the natural waters was inaccurately measured at low pH due to the failure to achieve lower pHs during the monochloramine contact time. Due to poor pH control during the initial pH 6.0 experiments, and the tendency of the pH to drift towards higher pH during the monochloramine contact period, the magnitude of the pH effect may have been underestimated. Based on the experience in this study, adjustment of pH by strong acid addition was not a satisfactory method of achieving lower pH in natural waters for the purposes of measuring a synergistic effect in the laboratory. Perhaps a better approach would be to obtain waters that were naturally at a pH close to 6.0 or to add a buffer to maintain pH at 6.0. Adding a buffer was not adopted as a method for

maintaining a stable pH because it was feared that the water quality might be changed during this process.

Li et al. (2000b) conducted their investigation of sequential inactivation of *C. parvum* oocysts with ozone followed by monochloramine in de-ionized water that was buffered at a single pH of 8.0, and, therefore, were not able to report on a pH effect. Rennecker et al. (2001) found that synergistic inactivation of *C. parvum* oocysts with ozone followed by monochloramine was independent of pH, however, they limited their study to the pH's 8 and 10.

Combined chlorine exists in the three equilibrium forms, monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3), however, monochloramine and dichloramine tend to dominate in water treatment conditions where excess ammonia is available. For a fixed Cl_2:N molar dose ratio, the proportion of monochloramine to dichloramine increases with pH and above pH 8 most of the combined chlorine is in the monochloramine form (Haas, 1999). Dichloramine has been shown to be a more effective anti-bacterial and anti-viral agent than monochloramine (Chang 1971). Monochloramine, however, is chemically more stable than dichloramine and this property may be of greater advantage in the inactivation of resistant microorganisms such as *C. parvum*. The more reactive dichloramine may be less able to diffuse through the *C. parvum* oocyst wall to reach sensitive targets within the oocyst interior.

5.7.3 Effect of Ozone Primary Treatment

A previous study in buffered de-ionized water (Li et al. 2001b) reported that the level of ozone primary treatment affected the magnitude of the synergistic effect, however, the nature of the relationship was not clear. In the present study with natural waters, ozone primary treatment had a significant impact on the synergistic effect of ozone followed by monochloramine sequential treatment in the natural waters. According to equation 5.2, a higher ozone primary treatment level yielded a greater synergistic effect.

One hypothesis for the mechanism of synergy is based on the premise that the rate of inactivation of *C. parvum* oocysts by weak oxidants such as monochloramine is limited by the rate at which the monochloramine molecule diffuses through the oocyst wall. Ozone reacts with lipids, proteins or other constituents of the oocyst wall, thereby weakening the oocyst wall and increasing it's permeability to monochloramine (Li et al. 2001b). A slightly different hypothesis supposes that ozone reacts rapidly with many of the same constituents within the oocyst wall and cavity that would otherwise consume monochloramine by reaction (Renneker et al. 2001). An increase in the level of synergistic effect with increasing ozone pre-treatment level, as observed in the present study, is consistent with either hypothesis. The results of this study, therefore, do little to confirm the mechanism of the synergistic effect. The observation that the synergistic effect is lower in natural water that is higher in turbidity, colour and total organic carbon, tends to support a hypothesis that the synergistic effect is related to reactions that occur at the surface of the oocyst wall rather within the interior of the oocyst.

5.7.4 Effect of Temperature

In this study with natural waters, the effect of temperature on synergy was found to be inconsequential. This unexpected finding was difficult to explain. However, it is possible that the effect of temperature may have been too small to be measurable relative to the variation inherent in the infectivity assay. In contrast, previous studies reported the synergistic effect of ozone and monochloramine sequential treatment on *C. parvum* oocysts to be temperature dependent. Li et al. (2001b) studied sequential inactivation at temperatures of 1°C, 10°C and 22°C, and reported that both gross inactivation and the synergistic effect increased with temperature. These researchers, however, assumed that k_1 (the monochloramine inactivation rate constant without ozone pre-treatment) was zero in their determination of the synergistic effect. The results of the present study (see the k_1 values presented in Tables 5.2 and 5.3) suggest that the Li et al. (2001b) approach may have underestimated the synergistic effect at low temperatures and overestimated the synergistic effect at higher temperatures. Driedger et al. (2001), on the other hand,

reported a stronger synergistic effect at lower temperatures. They found that the inactivation rate with monochloramine after ozone pre-treatment was 5 times faster at 20°C and 22 times faster at 1°C than the corresponding post-lag phase rates of inactivation with monochloramine treatment alone. This finding may be explained by their interpretation of the synergistic effect in terms of a ratio of monochloramine inactivation rates (i.e. k_2/k_1) versus an absolute difference in inactivation rates (k_2-k_1) in this study. The latter interpretation of a synergistic effect was preferred for this study because the ratio interpretation (k_2/k_1) becomes problematic and difficult to interpret when k_1 is either very close to zero or is negative.

CHAPTER 6: OZONE INACTIVATION OF *C. PARVUM* IN NATURAL WATERS

6.1 INTRODUCTION

A secondary objective of this study was to determine to what degree inactivation of oocysts by ozone alone, was affected by the natural water environment. An additional objective was to develop a *C. parvum* inactivation design criteria, using ozone in natural waters and compare the results measured in the natural waters to those obtained in previous studies (Li et al 2001b, Oppenheimer et al. 2000).

6.2 EXPERIMENTAL SETTINGS

The experimental conditions of the ozone inactivation trials of *C. parvum* in natural waters are shown in Table 6.1. All the conditions were investigated for the natural waters D and E, which varied significantly in colour, TOC, and turbidity. To estimate the temperature effect, experiments were conducted at two different temperatures (3 °C and 21°C). In most conditions specified in Table 6.1, data were collected for both high and low levels of ozone treatment. Inactivation levels of 1.7 log-units and 0.7 log-units were used to characterize the high and low level of ozone treatment targets, respectively. The *Ct* values were manipulated to achieve these two inactivation levels at each temperature based on the kinetic model predictions of Li et al. (2001b). To determine the effect of pH on the ozone disinfection kinetics, experiments were conducted at both pH 6 and 8 for most of the waters. For the experiments conducted at pH 6, waters A, B, C, D, and E, which had a natural pH of about 8, were adjusted to 6 by mineral acid addition prior to experiments and they were stable through out the ozone exposure period. Most of the trials were replicated twice under each of the conditions specified in Table 6.1. Ideally, randomization of the trials would have been a good approach for conducting these experiments. But this would have required storage of the natural waters for a long time (experimental period = 2 years), which could have changed the water quality

Table 6.1 Experimental conditions of the ozone inactivation trials with *C. parvum* done in the natural waters A to G

Natural waters	Experimental trials done?			
	21°C		3°C	
	pH 6	pH 8	pH 6	pH 8
A	Done	Done	Not done	Not done
B	Not done	Not done	Done	Done
C	Done	Done	Not done	Not done
D	Done	Done	Done	Done
E	Done	Done	Done	Done
F	Done	Not done	Done	Not done
G	Not done	Not done	Done	Not done

Done = Experiments were done under the conditions specified

Not done = Experiments were not done under the conditions specified

characteristics significantly. Hence, randomization of the trials was not done and the natural waters were used for experiments within a short period of 4 months after collection. The water quality analysis was done at the middle of the experimental period during these 4 months.

6.3 RESULTS AND ANALYSIS

Details of the ozone primary treatment conditions and the inactivation results are provided in Tables C.1 to C.7 of Appendix C. At 21°C, the experimental ozone *Ct* products were between 0.5 and 1.0 mg·min/L for the 0.7 log-unit ozone pre-treatment target and between 2.0 and 4.5 mg·min/L for the 1.7 log-unit pre-treatment target. At 3°C, the experimental ozone *Ct* products were between 5.0 and 9.0 mg·min/L for the 0.7

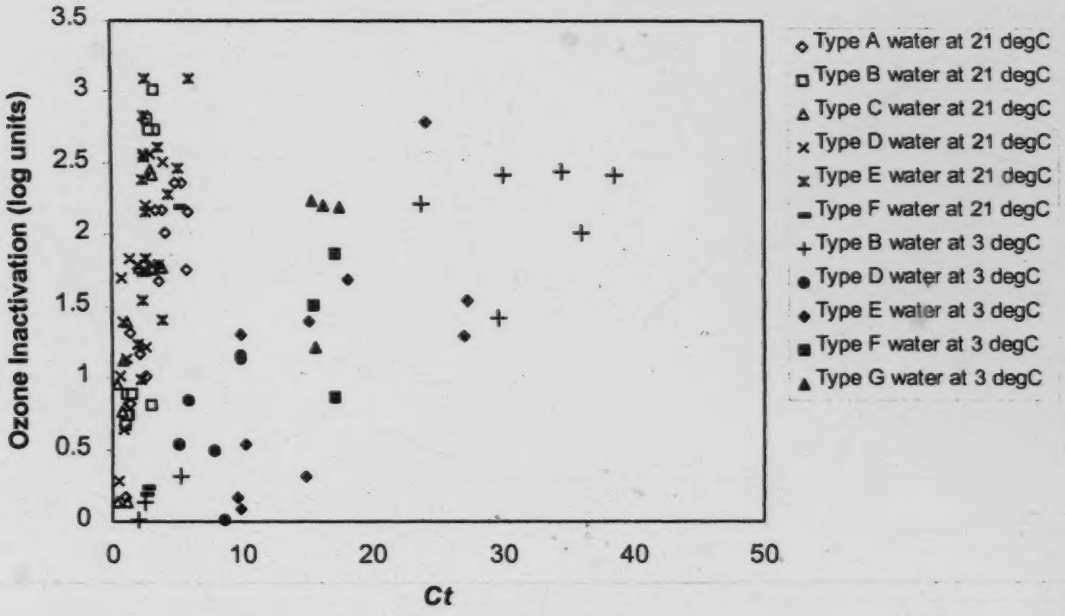

Figure 6.1 Ozone inactivation of *C. parvum* oocysts in natural waters at pH 6 to 8.

log-unit ozone pre-treatment target and between 10.0 and 27.0 mg·min/L for the 1.7 log-unit pre-treatment target. The inactivation of *C. parvum* oocysts exposed to ozone at 3°C (± 2°C) and 21 °C (± 0.4°C) in the natural waters are illustrated in Figure 6.1. The results in Figure 6.1 indicate a high variability in the data set for both high and low temperatures. However, visually, the inactivation data tend to exhibit a linear relationship at the high and low temperatures unlike the typical nonlinear relationship (I.g.H model) in buffered de-ionized water (Gyürék et al. 1999, Li et al. 2001b) characterized by an apparent shoulder, followed by a fast decline, and then a tail.

6.3.1 Control Trials

The reduction in infectivity in the control trials ranged from –1.0 to 1.0 log-units but was mostly within –0.6 to 0.2 log-units (Table 6.2). This range was slightly greater than the –0.7 to 0.7 log-units range that is typical for untreated control samples when using the neonatal CD-1 mouse assay in earlier studies with buffered de-ionized water (Li et al. 2001b). Histograms showing the reduction in infectivity of the oocysts in the control reactors for each natural water, are presented in Appendix E. The mean inactivation in the positive controls was 0.15 log-units indicating that exposure to the experimental water matrices and the various processing steps had little effect on mean oocyst infectivity. This also suggests that exposure of the oocysts to natural waters alone had little effect on oocyst infectivity, but may have increased the variability in the infectivity assay compared to the buffered de-ionized water. The control trials served primarily as a quality assurance for the experiments.

Table 6.2: Mean infectivity reduction of all the control trials in natural waters

Natural Water	Mean (log-units)	Standard Deviation
A	0.23	0.39
B	0.23	0.25
C	0.11	0.29
D	-0.17	0.38
E	-0.064	0.42
F	-0.45	0.27
G	-0.61	0.71

6.3.2 Comparison of the Results with I.g.H Model Predictions

The observed ozone inactivations were compared to I.g.H. model (Gyürék et al. 1999, Li et al. 2001b) predicted inactivations for equivalent ozone treatment conditions in this study. Predictions of *C. parvum* inactivation were generated for each experimental trial by substituting measured ozone exposure parameters (i.e. C_0, k_d) into the I.g.H. model given by equation 3.8. The I.g.H. model predictions generated using parameters provided by Li et al. (2001b) are valid for the pH range of 6 to 8. Within this range, Li et al. (2001b) found that the kinetic model parameters were largely unaffected by pH. Values of k_d, the first-order ozone decay coefficient, were determined for each experimental trial by fitting the equation $C = C_0\exp(-k_d t)$ to the measured ozone concentration-time profiles.

Comparisons between I.g.H. model predictions and the measured inactivation in the different natural waters after ozone treatment alone are shown in Figures 6.2 and 6.3. The central diagonal line in these figures represents a perfect model fit, while the upper and lower diagonal lines represent approximate upper and lower 90% confidence bands on the model predictions that were estimated by Gyürék et al. (1999) and Li et al. (2001b). These are, respectively, ± 0.7 log-units at 21°C and ± 0.6 log-units at 3°C.

Results with natural waters A, B, and C at 21°C indicated a reasonable match between the measured inactivation and I.g.H. model predictions (Figure 6.2 a). With the exception of a cluster of data points at a predicted infectivity reduction of 1.5 log-units, most of the experimental results were within ± 0.7 log-units of the model predictions. Model predictions for natural water B at the lower temperature also matched the measured inactivation reasonably well (Figure 6.3).

With natural waters D and E at 21°C, there appears to be some degree of lack-of-fit of the I.g.H. model at both the higher temperature (Figure 6.2b) and at the lower temperature (Figure 6.3). In some cases the model over-predicted inactivation by up to 1.0 log-unit. With natural waters F and G there seems to be a lack-of-fit except for Type F water at 5°C (Figure 6.3). The average model errors for each of the waters are shown in

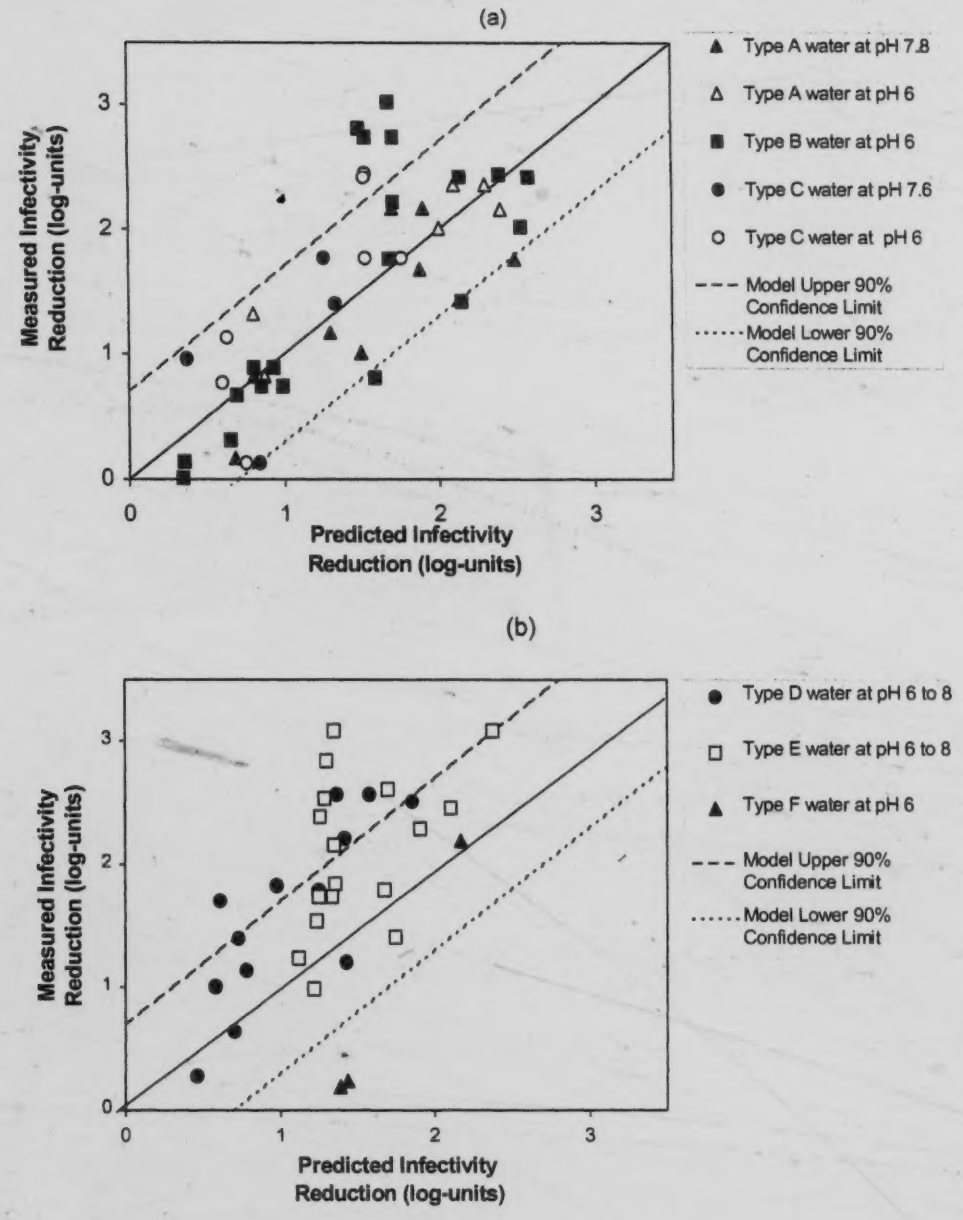
(a)
Measured Infectivity Reduction (log-units)
Predicted Infectivity Reduction (log-units)
Type A water at pH 7.8
Type A water at pH 6
Type B water at pH 6
Type C water at pH 7.6
Type C water at pH 6
Model Upper 90% Confidence Limit
Model Lower 90% Confidence Limit
(b)
Measured Infectivity Reduction (log-units)
Predicted Infectivity Reduction (log-units)
Type D water at pH 6 to 8
Type E water at pH 6 to 8
Type F water at pH 6
Model Upper 90% Confidence Limit
Model Lower 90% Confidence Limit

Figure 6.2 Comparison of measured infectivity reductions by ozone to Incomplete Gamma Hom (I.g.H) kinetic model predictions at 21 °C and pH values of 6 to 8 in (a) waters A, B, C and (b) waters D, E, and F.

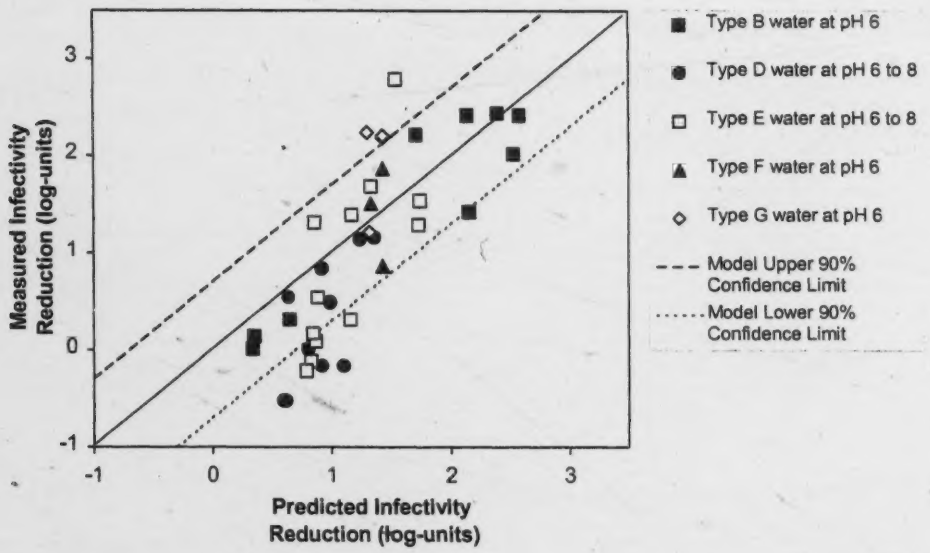

Figure 6.3 Comparison of measured infectivity reductions by ozone to Incomplete Gamma Hom (I.g.H) kinetic model predictions at 3°C and pH values of 6.0 to 8.0 in the natural waters B, D, E, F, and G.

Table 6.3. The lack-of-fit for the model predictions was observed in 4 of the 7 natural waters. In general, the cause of these deviations from model predictions is uncertain. Because the deviation was noted for natural waters D, E, F, and G but to a lesser extent with natural waters A, B, and C, it would seem that a water quality effect is unlikely. A more plausible explanation is batch-to-batch variation in oocyst sensitivity to ozone. Experiments conducted in Part I (Types A, B, and C), Part II (Types D and E), and Part III (Types F and G) were conducted in different experimental periods. Hence, different

batches of oocysts with potentially different resistance to ozone were used in the Part I, Part II, and Part III experiments. The oocyst batches can have different resistance to ozone because each batch of oocysts came from different hosts and hence they were exposed to different environments during their life cycles.

Table 6.3 Comparison of the I.g.H. model prediction versus the measured *C. parvum* inactivation by ozone

Natural Water	Temperature (°C)	[a]Average Model Error	[b]Significantly different from zero?
A	21	0.05 ± 0.37	No
B	21	-0.35 ± 0.74	No
C	21	-0.22 ± 0.51	No
D	21	-0.38 ± 0.64	No
E	21	-0.6 ± 0.58	Yes
F	21	0.79 ± 0.7	Yes
B	3 ± 2	0.16 ± 0.39	No
D	3 ± 2	0.64 ± 0.49	Yes
E	3 ± 2	0.25 ± 0.69	No
F	5	-0.02 ± 0.52	No
G	5	-0.59 ± 0.48	Yes

[a]Average of $[\hat{Y} - Y]$ ± Standard Deviation

where $\hat{Y}$ = I.g.H model prediction and Y = Measured Inactivation

[b]If zero lies between the upper and lower level of the average model error, then the model error is not significant

6.3.3 Chick-Watson Model and Design Criteria

The I.g.H. model is a complex model. The application of a simple model is always preferable to a more complex model with a large number of empirical parameters because it minimizes the prediction error of the model (Oppenheimer et al. 2000). Model evaluation demonstrated earlier (Oppenheimer et al. 2000) found that the higher parameter models did not result in lower *Ct* values to achieve a target level of *C. parvum* inactivation. The linear Chick-Watson model was found to be adequate for describing the inactivation kinetics in natural waters. In this study the general linear appearance of the inactivation data at temperatures of 3°C and 21°C in Figure 6.1 suggests that a linear model may be able to describe the ozone inactivation kinetics in natural waters.

Recalling from equation 3.7 that the Chick-Watson model can be described as

$$\ln\frac{N_0}{N} = kCt \qquad \text{Equation 6.1}$$

where $\ln(N_0/N)$ is the ozone inactivation in terms of log units, k is the inactivation rate constant (min^{-1}), C is the average ozone concentration (mg/L), and t is the time in minutes. For natural waters, k is expressed as a function of temperature (T), *water quality*, *oocysts batch*, and *oocysts age*:

$$k = K(\theta)^{T}(WaterQuality)^{a}(b)^{B}(c)^{D} \qquad \text{Equation 6.2}$$

where K, a, θ, b, and c are constants to be determined by regression analysis. In order to quantify the batch-to-batch variation of the oocysts resistance to ozone, the parameter "B" (for a particular batch and temperature, it is the average of all the trials of the ozone inactivation of the oocysts per unit ozone Ct) was used as a variable in equation 6.2. The ages of the oocysts were represented by "D".

Assuming n=1 and putting equation 6.2 in equation 6.1 the following relationship can be obtained:

$$\ln(N_0 / N) = K(\theta)^T (WaterQuality)^a (b)^B (c)^D Ct \qquad \text{Equation 6.3}$$

Let $Y = \ln(N_0/N)/(Ct)$

Hence equation 6.3 can be written as

$$Y = K(\theta)^T (WaterQuality)^a (b)^B (c)^D \qquad \text{Equation 6.4}$$

Taking the natural logarithim on both sides of equation 6.4 we get

$$\ln(Y) = \ln(K) + T\ln(\theta) + a\ln(WaterQuality) + B\ln(b) + D\ln(c) \qquad \text{Equation 6.5}$$

Equation 6.5 is a model like equation 3.9 where ln (Y) is the dependent variable and ln (K), T, ln (*Water Quality*), B, and D are the independent variables. A summary of the ozone inactivation data including the batch ozone inactivation per unit Ct and the age of the oocysts before experiments, is shown in Table 6.4. For the water quality a sufficient range of values (Table 3.3) was available to investigate eight water quality parameters: pH, conductivity, TDS, hardness, alkalinity, TOC, colour, and turbidity. However, a number of these parameters are highly correlated with each other and it is also unknown which of these parameters significantly influences the ozone inactivation kinetics of *C. parvum* in natural waters. Ideally, factorial analysis (Box et al. 1978) would be used in both the selection of experimental conditions and analysis of the data. However, due to the limited size of the database, it was not possible to evaluate all eight of these water quality parameters simultaneously in a 2^8 full factorial design (256 conditions). Hence, an alternative approach was adopted. A "water quality" index was developed. This index represented each of these eight water quality parameters individually. Each water quality index was used as a measure of water quality in the above regression equation 6.5. Multiple regression analysis of the data set shown in Table 6.3 was then done, by adopting the "backward elimination" (Draper and Smith 1966) approach using the regression tool in Microsoft Excel 2000. Model terms were rejected or retained based on

Table 6.4 Summary of the ozone inactivation data at their respective conditions

Water Type	Ozone Inactivation (log)	*Ct* (mg·min/L)	ln Y [Ln(Ozone Inactivation)/*Ct*)] [ln{log/(mg·min/L)}]	T (°C)	pH	Batch O_3 inactivation/*Ct* (B) [log/(mg·min/L)]	Oocysts Age (D) (days)
A	2.17	3.85	-0.57	22	7.8	0.509	38
A	1.17	2.19	-0.63	22	7.8	0.509	39
A	1.01	2.63	-0.96	22	7.8	0.509	40
A	2.17	3.42	-0.46	22	7.8	0.509	41
A	0.17	0.94	-1.71	22	7.8	0.509	52
A	0.82	1.20	-0.38	22	7.8	0.509	53
A	1.68	3.64	-0.77	22	7.8	0.509	66
A	1.76	5.70	-1.17	22	7.8	0.509	67
A	2.01	4.16	-0.73	22	6	0.509	68
A	2.36	5.35	-0.82	22	6	0.509	69
A	0.82	1.35	-0.50	22	6	0.509	122
A	2.36	4.85	-0.72	22	6	0.509	123
A	1.32	1.32	0.00	22	6	0.509	124
A	2.16	5.87	-1.00	22	6	0.509	125
B	0.81	3.03	-1.32	21	6	0.714	4
B	2.74	3.40	-0.22	21	6	0.714	5
B	1.76	3.15	-0.58	21	6	0.714	15
B	2.81	2.78	0.01	21	6	0.714	27
B	2.74	2.86	-0.04	21	6	0.714	28
B	3.02	3.29	-0.08	21	6	0.714	29
B	0.74	1.26	-0.53	21	6	0.714	30
B	0.67	0.94	-0.34	21	6	0.714	41
B	0.74	1.06	-0.36	21	6	0.714	42
B	0.89	1.18	-0.28	21	6	0.714	43
B	0.89	1.52	-0.53	21	6	0.714	44
C	2.45	3.01	-0.21	21	6	0.682	20
C	1.13	0.88	0.25	21	6	0.682	20
C	2.42	3.08	-0.24	21	6	0.682	22
C	1.77	2.17	-0.20	21	7.6	0.682	35
C	1.77	3.83	-0.77	21	6	0.682	49
C	0.13	1.14	-2.17	21	6	0.682	63
C	0.13	0.39	-1.09	21	7.6	0.682	75
C	1.77	3.08	-0.56	21	6	0.682	76
C	0.77	0.81	-0.05	21	6	0.682	77
C	1.4	1.17	0.18	21	7.6	1.347	41
C	0.96	0.42	0.83	21	7.6	1.347	41
D	1.79	1.96	-0.09	21	8.1	1.347	58
D	1.83	1.37	0.29	21	8.1	1.347	83
D	2.57	2.94	-0.13	21	8.1	1.347	85
D	2.57	2.44	0.05	21	8.1	1.347	97
D	1.7	0.72	0.86	21	6	1.347	99
D	0.28	0.48	-0.53	21	8.1	1.347	111
D	1.4	0.93	0.41	21	6	1.347	113
D	1.14	1.09	0.05	21	6	0.870	47
D	0.64	0.89	-0.33	21	8.1	0.870	77

(Continued)

Table 6.4 (Continued)

Water Type	Ozone Inactivation (log)	*Ct* (mg·min/L)	ln Y [Ln(Ozone Inactivation)/*Ct*)] [ln{log/(mg·min/L)}]	T (°C)	pH	Batch O_3 inactivation/*Ct* (B) [log/(mg·min/L)]	Oocysts Age (D) (days)
D	1.01	0.66	0.43	21	8.1	0.870	88
D	2.51	3.98	-0.46	21	6	0.870	102
D	1.21	2.69	-0.80	21	6	0.870	116
D	2.21	2.67	-0.19	21	6	0.870	117
E	1.41	3.85	-1.01	21	8.1	0.710	10
E	0.99	2.30	-0.84	21	6	0.710	24
E	3.09	6.00	-0.66	21	6	0.710	35
E	1.79	3.65	-0.71	21	8.1	0.710	36
E	1.24	2.01	-0.49	21	6	0.710	77
E	1.74	2.57	-0.39	21	8.1	0.710	78
E	2.29	4.37	-0.65	21	6	0.710	79
E	1.84	2.66	-0.37	21	6	0.710	91
E	2.46	5.07	-0.72	21	6	0.710	92
E	2.61	3.65	-0.34	21	8.1	0.710	94
E	2.84	2.49	0.13	21	8.1	0.710	105
E	3.09	2.68	0.14	21	8.1	0.710	106
E	2.16	2.65	-0.21	21	6	0.710	120
E	2.54	2.47	0.03	21	8.1	0.710	121
E	2.39	2.37	0.01	21	6	0.710	121
E	1.54	2.33	-0.41	21	8.1	0.710	122
E	1.74	2.33	-0.29	21	8.1	0.710	122
F	0.24	2.79	-2.45	21	6.3	0.280	111
F	2.19	5.25	-0.88	21	6.3	0.280	112
G	3.19	5.96	-0.63	21	5.8	0.280	123
F	0.19	2.62	-2.62	21	6.3	0.280	124
B	2.44	34.52	-2.65	2.5	6	0.059	22
B	2.02	36.06	-2.88	3	6	0.059	23
B	0.31	5.25	-2.83	2.8	6	0.059	24
B	2.22	23.77	-2.37	1.1	6	0.059	37
B	0.14	2.46	-2.86	1.1	6	0.059	38
B	0.01	1.98	-5.29	3.1	6	0.059	50
B	2.42	30.01	-2.52	2.9	6	0.059	51
B	1.42	29.65	-3.04	2.9	6	0.059	52
B	2.42	38.56	-2.77	2.9	6	0.059	53
D	0.54	5.16	-2.26	2.6	8.1	0.108	49
D	0.49	7.90	-2.78	5	6	0.108	91
D	1.14	9.86	-2.16	5	8.1	0.108	104
D	0.84	5.91	-1.95	7	6	0.108	118
D	1.16	9.90	-2.14	7	8.1	0.108	119
D	0.01	8.66	-6.76	1	6	0.058	21
E	0.31	14.88	-3.87	1	8.1	0.058	22
E	0.09	9.88	-4.70	1	8.1	0.058	23
E	1.29	26.98	-3.04	1	6	0.058	37
E	1.54	27.26	-2.87	1	6	0.058	52
E	1.69	18.14	-2.37	1	8.1	0.058	64

(Continued)

Table 6.4 (Continued)

Water Type	Ozone Inactivation (log)	*Ct* (mg·min/L)	ln Y [Ln(Ozone Inactivation)/*Ct*)] [ln{log/(mg·min/L)}]	T (°C)	pH	Batch O_3 inactivation/*Ct* (B) [log/(mg·min/L)]	Oocysts Age (D) (days)
E	0.17	9.58	-4.03	1	8.1	0.058	65
E	0.54	10.28	-2.95	1	6	0.058	93
E	1.31	9.89	-2.02	1	6	0.058	107
E	2.79	24.08	-2.16	1	6	0.058	108
E	1.39	15.12	-2.39	1	8.1	0.058	119
F	1.87	17.10	-2.21	5	6.3	0.106	113
F	1.51	15.44	-2.32	5	6.3	0.106	114
F	0.87	17.10	-2.98	5	6.3	0.106	125
G	2.19	17.55	-2.08	5	5.8	0.106	126
G	2.21	16.27	-2.00	5	5.8	0.106	137
G	2.24	15.32	-1.92	5	5.8	0.106	138
G	1.21	15.68	-2.56	5	5.8	0.106	138

the statistical significance of the coefficients at the 90% confidence level. Since the variable "water quality" in equation 6.5 was represented by eight individual water quality parameters separately, eight separate multiple regression analysis were done. This approach did not permit the effect of the simultaneous consideration of all the water quality parameters on the ozone inactivation kinetics of *C. parvum*. Nevertheless, the analysis gave valuable information about the effect of the dominating water quality factors on the inactivation kinetics. For the water quality parameters "TOC" and "Colour" the values were not available for waters (A) and (B and C) respectively (Table 3.3). Hence for the regression analysis, the inactivation data of the corresponding waters were not used. The water quality parameters having values which are "below detection level" (Table 3.3), were set at the detection limit of the data points and were used for the multiple regression analysis.

The multiple regression analysis indicated that none of the individual eight water quality parameters were statistically significant at the 90 % confidence level ($p > 0.10$). According to the multiple regression analysis, only the variables *T*, *B*, and *D* were found to be statistically significant at the 90% confidence level. The variable "*water quality*" was found to be insignificant for all the eight water quality parameters in the eight-regression analysis. The linear model obtanined after regression analysis is given by:

$$\ln(Y) = -3.52 + 0.083T + 1.398B + 0.0038D \qquad \text{Equation 6.6}$$

Given the computed values of the ANOVA *F*-statistic and the *p*-values for each parameter, the overall model and the individual parameters were significant at the 90% level. The r^2 and the standard error of the above model, was determined to be 0.76 and 0.68, respectively.

A similar regression analysis was repeated for equation 6.5 but this time without considering the effect of the oocysts batch-to-batch variation (B) and oocysts age (D). Multiple regression analysis of the data set shown in Table 6.4 was once again done, by adopting the "backward elimination" and model terms were rejected or retained based on the statistical significance of the coefficients at the 90% confidence level. Once again all the variables of *water quality* were found to be insignificant and equation 6.5 now reduced to the following equation:

$$\ln(Y) = -3.26 + 0.13T \qquad \text{Equation 6.7}$$

The r^2 and the standard error of this model, was determined to be 0.69 and 0.76, respectively. On comparing equations 6.6 and 6.7, it was found that removing the effect of the variables *B* and *D* from equation 6.6 yielded an equation 6.7 with a marginal deterioration in the r^2 and standard error of the model. This showed that equation 6.6 was slightly better in model prediction than equation 6.7. However, a design criteria based on oocysts resistance to ozone in each batch, and oocysts age would be of limited practical significance as there is no way of determining them, beforehand. Hence, the more rational approach for developing a model for predicting the relationship between inactivation and *Ct* values is to elimnate "batch" or "lot" and "age" as a variable in the model (Clark et al. 2003). Batch-to-batch variability should rather be included in the general variability of the model and accounted for by using a safety factor. Thus equation 6.7 instead of equation 6.6 was used to develop an ozone inactivation of *C. parvum* design-criteria.

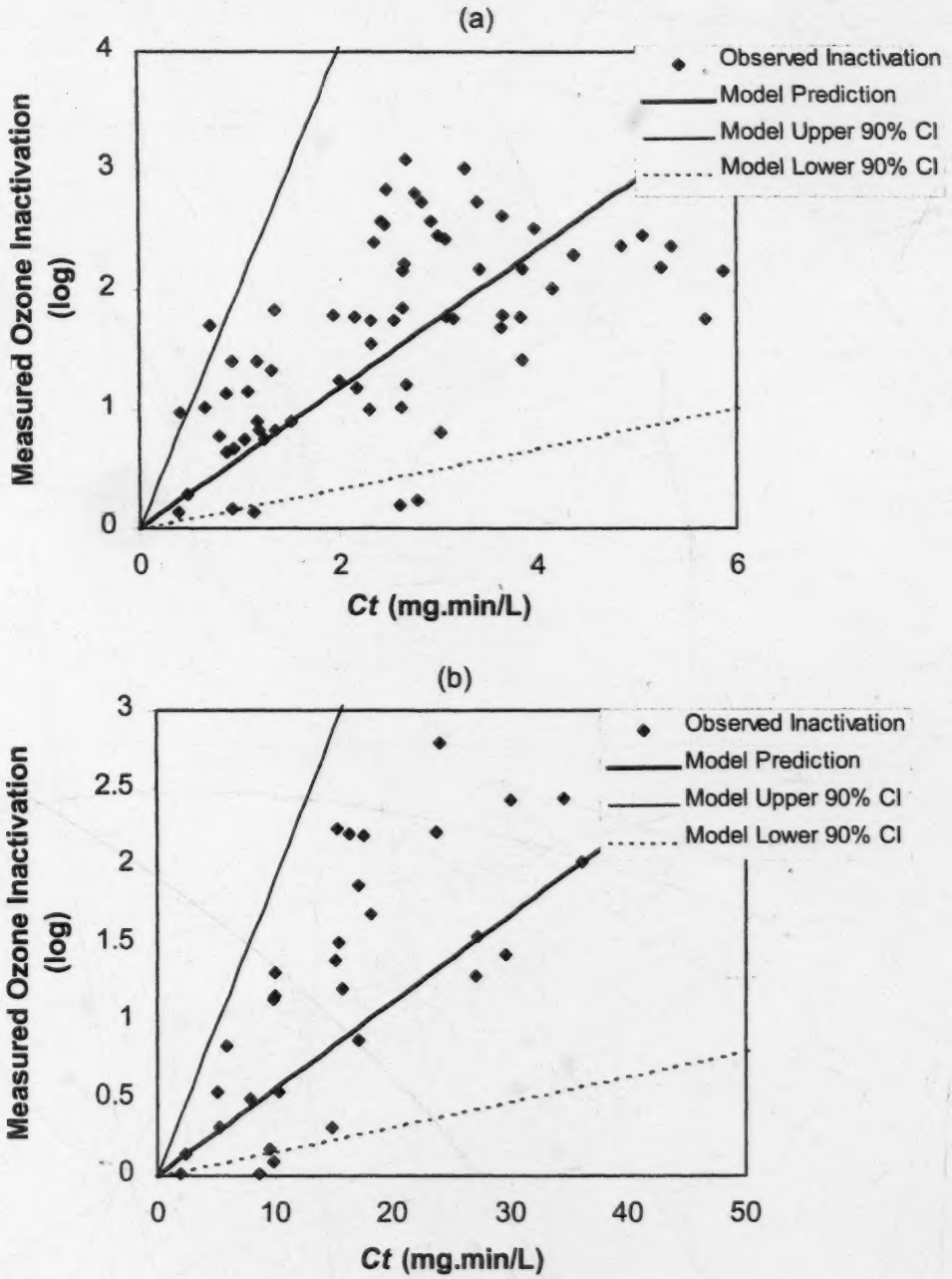

Figure 6.4 Fit of the Chick-Watson model for predicting the ozone inactivation of *C. parvum* oocysts in natural waters A to G at pH 6 to 8 and temperatures of (a) 21 °C and (b) 3 °C.

Table 6.5: Comparison of the Chick-Watson model prediction versus the measured *C. parvum* inactivation by ozone

Natural Water	Temperature (°C)	[a]Average Model Error	[b]Significantly different from zero?
A	21	-0.69 ± 0.98	No
B	21	0.01 ± 0.65	No
C	21	0.10 ± 0.62	No
D	21	0.56 ± 0.68	No
E	21	0.25 ± 0.97	No
F	21	-1.52 ± 0.15	Yes
B	3 ± 2	-0.02 ± 0.47	No
D	3 ± 2	0.14 ± 0.41	No
E	3 ± 2	0.36 ± 0.64	No
F	5	0.03 ± 0.53	No
G	5	0.40 ± 0.48	No

[a]Average of $[\hat{Y} - Y]$ ± Standard Deviation

where $\hat{Y}$ = Chick-Watson model prediction and Y = Measured Inactivation

[b]If zero lies between the upper and lower level of the average model error, then the model error is not significant

Reformulating equation 6.7 after substituting Y yields the following equations:

$$\ln(N_0 / N) = 0.038(1.139)^T Ct \qquad \text{Equation 6.8}$$

The developed Chick-Watson model in equation 6.8 was used to fit the ozone *C. parvum* inactivation data obtained in this study. Fitted (predicted) infectivity reductions for the model parameter estimates are plotted with respect to observed infectivity reductions in Figure 6.4. The 90 % upper and lower bound inactivation rates are also plotted using the procedures described elsewhere (Clark et al. 2002). The Chick-Watson model exhibited both underestimation and overestimation of the observed (true) level of *C. parvum* inactivation both at high and low temperatures. In some cases the model underestimated the inactivation as high as 1.5 log-units and 0.75 log-units at 21°C and 3°C, respectively. The model also overestimated the predictions as high as 1.5 log-units and 0.5 log-units at 21°C and 3°C, respectively. The average model errors for all the natural waters are shown in Table 6.5. The results presented in table 6.5 indicated that, except for Type F water, the model errors were not significantly different from zero. This indicates a reasonable match between the measured inactivation and Chick-Watson model predictions. The failure to predict the oocysts inactivation in Type F water may be due to the batch-to-batch variation of the oocysts or the age of the oocysts, which were not considered in the model. Based on the above Chick-Watson model, the *Ct* products were calculated for 1, 2, and 3 log-units of *C. parvum* inactivation in natural waters at temperatures 1°C,

Table 6.6: *Ct* requirements for ozone inactivation of *C. parvum* at various conditions based on the Chick-Watson model developed for natural waters A to G (no safety factor).

Target Inactivation (log-units)	*Ct* requirements (mg×min/L)		
	1 °C	10 °C	20 °C
1.0	23	7.2	1.9
2.0	46	14.3	3.9
3.0	69	21.5	5.8

10°C, and 20°C respectively (Table 6.6) and this was used to develop a design criteria. The high uncertainty in the inactivation data set should be accounted for by using a safety factor in practical applications. This would be highly prudent from a public health perspective (Clark et al. 2002). However, safety factor is a matter of choice and judgement. An approach commonly used is by incorporating a safety factor (Clark et al. 2002) in the *Ct* requirements for a given level of confidence interval (say 90 %). The 90 % confidence interval band of the *Ct* requirements shown in Figure 6.4 of this study suggests that adopting this approach may be suitable at lower inactivation levels (below 1 log inactivation). But it is unnecessarily conservative at higher inactivation levels. However, from the data set (Figure 6.4) it appears that a safety factor of 1.5 times the *Ct* requirements in Table 6.6 will give enough cushion of safety for *C. parvum* inactivation in the natural waters under the studied conditions.

6.4 DISCUSSIONS

The inactivation of *Cryptosporidium parvum* oocysts using ozone was found to be feasible in the studied natural waters. However, there was high variability in the data set. The cause of the high variability in the data set is not known. However, the precision of the infectivity determination using the animal infectivity assay might have been reduced in natural waters compared to buffered de-ionized waters due to the presence of dissolved organic compounds and other substances.

6.4.1 Comparison with Previous Studies

Oppenheimer et al. (2000) studied ozone inactivation of *C. parvum* oocysts in natural waters and used mouse infectivity as the viability assay. The *Ct* requirements they estimated were 16 and 31 mg·min/L for 1 and 2 log-units inactivation at 3°C respectively; and 2.3 and 3.5 mg·min/L for 2 and 3 log-units of inactivation at 21°C respectively. These results were approximately 1.5 times lower than the *Ct* requirements found in this study.

In buffered de-ionized water (Li et al. 2001b), the *Ct* requirements were estimated to be 15.3 to 17 and 41.9 to 43.1 mg·min/L for 1 and 2 log-unit inactivation at 1°C respectively; and 4.6 to 4.7 and 8.0 to 8.3 mg·min/L for 2 and 3 log-units inactivation at 21°C respectively. These results were approximately 1.5 times lower than this study at 1°C. On the other hand at 21°C, the *Ct* requirements in the buffered de-ionized water were about 1.5 times higher than this study.

The overall comparison indicates that the *Ct* requirements in this study were comparable to that of the earlier studies. At 21°C, the ozone *Ct* requirements in natural waters of this study were close and comparable to that of the earlier studies. However, at lower temperatures, the *Ct* requirements in this study were much higher than all the previous studies. This suggested that previously published data obtained for laboratory de-ionized water and natural waters underestimate the ozone *Ct* requirements in natural waters of this study at low temperatures. This also indicated that for the studied natural waters it may be practically difficult to achieve the target level of inactivation of *C. parvum* oocysts at low temperatures due to the high level of ozone *Ct* requirements. Nevertheless, it indicates that the ozone inactivation observed earlier in buffered de-ionized water is not inhibited significantly in the studied natural waters.

6.4.2 Effect of Water Quality

Based on the results of this study none of the water quality parameters individually had an effect on the ozone inactivation kinetics of *C. parvum* in the natural waters. This also indicated that none of the water quality parameters have a dominating effect on the inactivation kinetics under the studied conditions. However, each natural water, represents a unique water quality matrix and the simultaneous consideration of all the water quality parameters might have an effect on the inactivation kinetics at some level. While insufficient to conclude, it appears from the results, that for the 7 studied natural waters the ozone inactivation of *C. parvum* is not inhibited.

6.4.3 Effect of Temperature

For every 10°C rise in temperature, the inactivation rate constant (k) was found to increase by a factor of 3.7, in this study. According to Oppenheimer et al. (2000), the inactivation rate constant increased by a factor of 4.5 for each 10°C rise in temperature. Earlier studies in buffered de-ionized water (Li et al. 2001b) indicated that the results conform to classical thermodynamics in which the inactivation rate constant roughly doubles for every 10°C temperature rise. The results of this study in natural waters indicate a certain departure from the findings of both the earlier studies. This also indicated that the influence of water temperature on the ozone inactivation kinetics for *C. parvum* is greater than the earlier study in buffered de-ionized water but less than the earlier study in natural water.

6.4.4 Oocyst Batch and Oocyst Age

Previous studies (Rennecker et al. 2000a; Rennecker et al. 2001; Corona-Vasquez et al. 2002) have shown that oocysts from different batches, or even from the same batch but of different age, can have different resistance to ozone inactivation. Hence, the kinetics of ozone inactivation can vary both with the oocysts batch and oocysts age. The results of this study indicated a similar dependence of batch-to-batch variation of oocysts resistance to ozone, on the inactivation kinetics. Equation 6.6 indicated a statistically significant effect of the batch-to-batch variation of the oocysts resistance to ozone. This supports the earlier hypothesis (Corona-Vasquez et al. 2002) that oocysts batches could include "weak" oocysts, "strong" oocysts, or a certain mixture of "weak" and "strong" oocysts, and that the inactivation rate constants for these oocysts were unique at a given temperature. Equation 6.6 also indicated a statistically significant effect of the age of the oocysts on the ozone inactivation kinetics. An increase in the age of the oocysts resulted in an increase in the oocyst inactivation level per unit *Ct*. This indicated that the oocysts resistance to ozone decreased with time.

CHAPTER 7: GENERAL DISCUSSION

7.1 SYNERGISM AND ITS' IMPLICATIONS

Synergistic processes in nature have often been the driving force for research to understand many environmental phenomena. The first observation of synergism is often regarded as an interesting phenomenon, which is beyond the understanding by normal scientific explanations. Synergistic effects may have serious implications as it may cause a highly detrimental or highly beneficial effect in the environment.

In this study, the synergistic effect was used as a tool for measuring the performance of microorganism reduction during water treatment by sequential treatment with chemical oxidants. One of the driving forces behind this study was its' huge implications for the water treatment industry. In drinking water utilities sequential treatment of water using chemical oxidants is common and has been used as a treatment strategy for many years. However, the possibility of the existence of synergistic effect on the inactivation of waterborne parasites had not been investigated rigorously. If a significant amount of synergistic effect exists, it may result in a number of benefits some of which may be as follows:

1. reduction in the applied dose of the chemical oxidants in water for achieving a certain level of microorganism reduction;
2. reduction in the contact time of the chemical oxidants in water for achieving a certain level of microorganism reduction;
3. reduction of the formation of disinfection-by-products in water due to the lower chemical oxidant dose;
4. reduction in the operating cost of water treatment.

The implications mentioned above justifies that "synergism" may have a huge impact in the water treatment industry in general. The understanding of synergistic effect will also

help us to fundamentally understand the concept of synergism in water treatment and open new areas of research.

7.2 PROBLEM REVISITED

The current challenges in water treatment have generated the need to explore more rigorously the synergistic inactivation of *Cryptosporidium* spp. oocysts in natural waters. The behavior of oxidants during *C. parvum* inactivation is more representative of actual waters in treatment plants. Microbial inactivation in natural waters using chemical oxidants presents a lot of complexity. The oxidants may react with dissolved, colloidal, and particulate matter, and these reactions might interfere with some of the reactions responsible for microbial inactivation. Further, water quality parameters like pH, alkalinity, hardness, total dissolved solids, total organic carbon, turbidity etc. may influence the microbial inactivation process to a significant extent. The questions that are critical issues during these processes are as follows:

1. Does synergistic effect of *C. parvum* inactivation in natural waters exist?
2. What combinations (ozone followed by chlorine; chlorine dioxide followed by monochloramine etc.) of oxidants exhibit synergistic effect in natural waters?
3. How does the level of primary oxidant treatment influence the synergistc effect?
4. How does pH, temperature, and water quality influence the synergistic effect?
5. If water quality significantly influences the synergistic effect, which water quality parameters are the true indicators of water quality as far as the synergistic effect is concerned?
6. How does the synergistic effect in natural waters compare with the synergistic effect achieved in earlier studies in buffered laboratory water?
7. If the synergistic effect in natural water exists, how can the results be utilized to develop reliable design criteria that can be used by the water treatment industry to guide facility design?

In this thesis, an attempt was made to answer some of the above questions.

Although a great deal of research has been done to test the sequential treatment of *C. parvum* using multiple disinfectants, the problem that remained was that synergism and the control of waterborne cryptosporidiosis remained a phenomenon that was poorly understood and may not be universally applicable. The present study helped us to understand some of the processes in natural waters but did not provide answers to some of the questions like:

1. Is there a minimum *Ct* value for the primary oxidant beyond which no synergistic effect is obtained?
2. What type of disinfectant by-products are produced when multiple oxidants are added and how do they affect the inactivation kinetics?
3. What are the mechanisms of sequential chemical inactivation at the cell level in natural waters?
4. Can the synergy demonstrated in *C. parvum* be extended to other pathogens like *Giardia* spp., *E. coli* etc. under similar conditions?

Nevertheless, from the results of this study, the water treatment industry will have better direction on how to implement new regulations that may include credit for synergy from sequential treatment. A better understanding of all the influencing factors like pH, temperature, and water matrix effects from the current and further studies may lead to the development of a more rigorous design framework for water utilities in the future.

7.3 IMPLICATIONS FOR WATER TREATMENT UTILITIES

7.3.1 Ozone Followed By Free Chlorine Sequential Treatment

In this study, the magnitude of the synergistic effect determined in the high pH natural water samples was between 3 and 42 times smaller than previously reported for buffered de-ionized water (Li et al. 2001b) at temperatures of 1°C and 21°C, respectively. Based on the computed mean synergistic effect in the high pH natural waters, after ozone

pretreatment, the free chlorine $C_{avg}t$ product required to produce a 1 log-unit synergistic effect is estimated to be 23 800 mg·min/L. Assuming a free residual chlorine concentration of 2 mg/L in the finished water, which is typical in the industry, the required contact time is more than 8 days. Clearly, this treatment strategy would be impractical for drinking water utilities since such a contact time would be difficult to achieve by most water treatment facilities.

For the low pH natural waters, the synergistic effect was found to be higher, compared to the high pH waters. For natural water F at 21°C, after 0.8 log-unit ozone pretreatment, the $C_{avg}t$ product required to produce a synergistic effect equivalent to 1 log-unit synergistic effect was estimated to be 1 098 mg·min/L. For a 2 mg/L chlorine residual, a contact time of 9 hours was required. This contact time may be achievable for certain facilities with sufficient finished water reservoir storage. Therefore, for low pH natural waters, a sequential treatment strategy may be a feasible approach to achieving additional protection against *C. parvum*. Hence, it is not recommended that ozone followed by free chlorine be used as a treatment strategy to achieve additional *C. parvum* inactivation credit in natural waters having a higher pH (8.0). But ozone followed by free chlorine may be used as a treatment strategy to achieve additional inactivation credit in natural waters having a low pH (6.0). However the synergistic effect must also be confirmed for the natural waters under consideration using site-specific testing.

In a recent survey, the mean and median pH of source waters in 171 large and medium-size water treatment facilities in the United States, were reported to be 7.48 and 7.55, respectively (AWWA Water Quality Division Disinfection Systems Committee, 2000). This indicates that most of the source waters in the drinking water utilities in North America have a higher pH. Since the findings of this study suggest that the synergistic effect can only be achieved in low pH source waters and it is practically difficult to achieve low pH during free chlorine treatment in high pH natural waters; it can be hypothesized that for many water treatment facilities it may be practically difficult to achieve a significant synergistic effect using this combination of chemical oxidants.

7.3.2 Ozone Followed By Monochloramine Sequential Treatment

For ozone followed by monochloramine sequential treatment, the magnitudes of the synergistic affect in the natural waters were such that they may reasonably be achieved in some water treatment situations. The *Ct* required for a 1 log-unit synergistic effect at 22°C and with 0.4 log-unit of ozone primary treatment was about 1 080 mg·min/L. For a monochloramine residual of 2 mg/L, the contact time required would be about 9 hrs. This contact time may be feasible for water utilities to achieve monochloramine contact time with sufficient reservoir capacity. Moreover, some level of gross inactivation and synergistic effect may be expected even in poorer quality waters containing suspended solids, colloidal material and dissolved organic material. The pH effect determined in this study indicates that a sequential treatment strategy with ozone followed by monochloramine will be effective for alkaline waters with above neutral pH. This is a distinct advantage over a sequential treatment strategy based on free chlorine rather than monochloramine.

Thus the use of ozone followed by monochloramine may be considered as a potentially feasible strategy for achieving synergistic inactivation credit for *C. parvum* in natural waters in the industry under certain conditions. A sequential disinfection strategy, however, should not be used to replace other barriers in the water treatment process that provide protection against *C. parvum* oocysts such as filtration or primary disinfection. Rather, sequential treatment may potentially be used by water utilities, which are seeking additional *C. parvum* inactivation credit over and above, what is provided by optimized filtration and primary disinfection. Because the magnitude of the synergistic effect in this study was found to be a function of water quality, it is recommended that any water supplier considering using a sequential disinfection strategy for achieving maximum public health protection verify the level of *C. parvum* inactivation in site-specific laboratory or pilot-scale testing.

7.3.3 Ozone Treatment Only

Previous studies with ozone in buffered de-ionized water found ozone to be a feasible treatment strategy for inactivating most waterbone microorganisms including *C. parvum*. However, very few studies investigated its' efficacy in natural waters (Oppenheimer et al., 2000). In this thesis, the earlier studies in natural waters were complemented by additional data in seven different types of natural waters. Ozone inactivation kinetics of *C. parvum* in the studied natural waters was determined. Ozone was found to be a feasible treatment strategy for the inactivation of *C. parvum* in natural waters. At lower temperatures, the *Ct* requirements in this study were much higher than the previous studies in buffered de-ionized water and natural waters. This indicated that for the studied natural waters it may be practically difficult to achieve the target level of inactivation of *C. parvum* oocysts at low temperatures due to the high level of ozone *Ct* requirements. A design criteria based on the results of this study can be used to develop a design strategy only for those utilities having source waters with similar water quality characteristics as the waters used in this study. However, the use of design criteria for a full-scale water treatment plant based on any disinfection studies alone should be done with caution, as there are a number of factors that may influence the data set. Over the last decade there have been several studies done in this field but it is difficult to make any direct comparisons between the results of these studies as different approaches and protocols were used by different laboratories for their studies. The differences in these studies were mainly due to the different methods (animal infectivity, in-vitro excystation, cell culture etc.) used for measuring inactivation, types of reactors (batch, continuous etc.), and also the method of application and measurement of the chemical oxidants. Hence, it causes a lot of confusion among water treatment professionals to interpret or extrapolate these results to develop design criteria for full-scale treatment plant. In this context the Badenoch report (1995) concluded the following (Oppenheimer et al. 2000):

" results from disinfection studies can be used only to give an indication of the relative efficacy of different disinfectants rather than firm criteria on which design could be based."

Nevertheless, these disinfection studies give a good indication about the efficacy of the process and an understanding of the inactivation kinetics under various situations. In the present study with ozone, more comprehensive data set is required to identify and measure the influence of the water quality parameters on the inactivation kinetics. In addition, in-situ bench-scale tests should be done to validate the model for specific conditions. In order to take into account the variations due to the oocyst batch, age, and the method of testing, the use of a safety factor is also highly prudent from a public health perspective.

7.4 FUTURE SCOPE OF SYNERGISM

The present study highlights the importance of synergism in water treatment. It also fills in some of the gaps in the information required for rational engineering of sequential inactivation of *C. parvum* in natural waters. It will be of significant importance for water utilities looking for options for getting credit for microorganism reduction. However, the problem remains that synergism is a phenomenon that is not fully understood and may not be universally applicable. Further studies in this area will not only help to understand synergism but also help to apply engineering concepts with confidence.

CHAPTER 8:CONCLUSIONS AND RECOMMENDATIONS

8.1 CONCLUSIONS

The synergistic inactivation of *Cryptosporidium parvum* oocysts in natural water samples using ozone followed by free chlorine and ozone followed by monochloramine was studied. The results of the study also permitted the study of the ozone inactivation kinetics of *C. parvum* in natural waters. The main conclusions of the study are listed below:

1. For the experimental ranges investigated, a synergistic effect was measured for sequential treatment of *C. parvum* oocysts with ozone followed by free chlorine in the seven natural waters tested. The measured synergistic effect in the five alkaline (pH 8) natural waters was statistically significant but was much smaller than previously reported for *C. parvum* oocysts exposed to this chemical combination in buffered de-ionized water. Temperature, ozone primary treatment level, and water type did not have measurable impacts on the synergistic effect. Given the limited magnitude of the synergistic effect and the large *Ct* values, the free chlorine contact time that would be required for a 1 log-unit synergistic effect in the high pH (8.0) natural waters tested is not practical for water treatment. Efforts to increase the synergistic effect in these natural waters by reducing the initial pH to 6 by acid addition were unsuccessful. In the two low-alkalinity (pH approximately 6) natural waters tested, the measured synergistic effect was much greater than in the alkaline waters, but was still less than that measured earlier in buffered de-ionized water. It was concluded that the reduction of the synergistic effect was in part due to the high pH and alkalinity of the natural waters tested, and in part due to other, unidentified natural water quality characteristics. Thus, sequential treatment with ozone followed by free chlorine may only be a feasible strategy for achieving synergistic *C. parvum* inactivation credit for water treatment facilities with low alkalinity natural waters having a pH near 6. The

size of the synergistic effect may also be a function of the specific characteristics of the water under consideration.

2. A statistically significant synergistic effect was measured for ozone followed by monochloramine sequential treatment in the natural waters under the studied conditions. The magnitude of the synergistic effect measured in the natural waters was also comparable to that previously reported for buffered de-ionized water. Statistically significant impacts of ozone primary treatment level, pH, and water quality on the monochloramine synergistic effect were measured. The synergistic effect increased with pH (initial pH 6 to 8.1), with the level of ozone pre-treatment (0.7 log-unit to 1.7 log-units), and with superior water quality in terms of TOC, colour, and turbidity, under the studied conditions. Due to the tendency of the pH of the natural waters to drift from 6 to 8 during experiments, the pH effect may have been underestimated. Temperature did not have a significant impact on the synergistic effect though it did affect the gross inactivation levels. Based on the statistical analysis, *C. parvum* inactivation design criteria in natural waters using ozone followed by monochloramine, were established. For those water treatment plants able to provide sufficient contact times, ozone followed by monochloramine may be a practical means of achieving additional *C. parvum* inactivation credit due to the synergistic effect.

3. The inactivation of *Cryptosporidium parvum* oocysts using ozone was found to be feasible in the studied natural waters. On comparing the results with the model predictions of the earlier developed Incomplete gamma Hom model in buffered de-ionized water, it was found that lack-of-fit of the model predictions was observed in 4 of the 7 natural waters. A linear Chick-Watson model was found to be adequate for describing the inactivation kinetics in the studied natural waters. The results with the Chick-Watson model indicated a statistically significant effect of temperature, batch-to-batch variation of the oocysts resistance to ozone, and the age of the oocysts on the inactivation kinetics of *C. parvum* using ozone. The individual water quality

parameters including pH did not affect the inactivation kinetics significantly. At lower temperatures, the *Ct* requirements in the studied natural waters were much higher than the previous studies in buffered de-ionized water and natural waters. This indicated that for the studied natural waters it may be practically difficult to achieve the target level of inactivation of *C. parvum* oocysts at low temperatures due to the high level of ozone *Ct* requirements. Based on the Chick-Watson model, ozone disinfection design criteria for the inactivation of *C. parvum* in natural water, were established.

8.2 RECOMMENDATIONS

With regard to the direction of future research on this topic, the following recommendations are made:

1. Most of the natural waters used in the study had a natural pH of 8.0 and only a few experimental trials were conducted with low pH (6.0) natural waters. Hence, additional studies in low pH natural waters are recommended. For ozone followed by free chlorine sequential treatment, the data obtained in this study were insufficient for determining the extent of synergy for low pH natural waters under more diverse conditions. Additional studies would help to establish whether the synergistic effect measured with the combination of ozone followed by monochloramine was present at lower pH. Additional studies would also help to determine the magnitude of the effect of other factors like ozone pre-treatment level, water quality, and temperature influencing the synergistic effect in low pH natural waters.

2. The importance of pH on the synergistic effect of ozone followed by monochloramine suggests that additional studies using natural waters in which the pH has been increased to levels exceeding pH 8 (i.e. pH 9 or 10) would be of practical interest. If the synergistic effect was more pronounced at these higher and untested pH levels, pH adjustment together with monochloramine treatment may be an attractive option.

3. Synergistic effect was found to increase with increasing ozone pre-treatment level for ozone followed by monochloramine sequential treatment. Additional studies should be done to investigate whether the synergistic effect observed increase continuously with increasing ozone pre-treatment level or whether the mechanism that accounts for the synergistic effect become saturated.

4. Additional studies should be done to determine the mechanisms of sequential chemical inactivation of the oocysts at the cell level in natural waters.

5. The results of the ozone exposure experiments indicated that the effect of ozone on *C. parvum* oocysts was a function of the batch-to-batch variation of the oocysts resistance to ozone. This raises the question of the potential for differential responses to different environmental strains of oocysts to chemical oxidant treatments. The present study, however, was not designed to isolate the effects of differences in oocyst strains, so this effect was uncertain. Hence, further research should be directed at determining the significance of strain-to-strain variations in oocyst response to chemical disinfectants.

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APPENDIX A

WATER QUALITY ANALYSIS OF THE NATURAL WATER SAMPLES USED IN EXPERIMENTS

Table A.1

Water quality analysis of the natural water samples used in experiments

Water Quality Parameter	Water A	Water B	Water C	Water D	Water E	Water F	Water G
Routine Water Analysis							
Chloride (Cl) [mg/L]	<1	2	92	31	6	2	<1
Nitrate+Nitrite-N [mg/L]	<0.1	0.1	4.3	1.7	0.2	<0.1	<0.1
pH	7.8	7.7	7.6	8.1	8.1	6.3*	5.8*
Conductivity (EC) [µS/cm]	294	175	604	498	496	26.7	15.8
Bicarbonate (HCO_3) [mg/L]	114	97	92	198	220	11	10
Carbonate (CO_3) [mg/L]	<5	<5	<5	<5	<5	<5	<5
Hydroxide [mg/L]	<5	<5	<5	<5	<5	<5	<5
Alkalinity, Total [mg/L]	93	79	75	162	181	9	8
Ion Balance (%)	101	106	103	104	98.9	Low EC	Low EC
TDS (Calculated) [mg/L]	171	89	348	290	279	13	9
Hardness [mg/L]	151	87	169	224	245	9	6
ICP metals							
Calcium (Ca) [mg/L]	37.2	23.6	41.5	51.1	67.9	2.9	2.0
Potassium (K) [mg/L]	0.7	1.4	3.9	3.4	1.1	<0.1	0.2
Magnesium (Mg) [mg/L]	14.2	6.8	15.8	23.4	18.4	0.5	0.2
Sodium (Na) [mg/L]	3	2	15.8	23	6	1	<1
Sulfate (SO_4) [mg/L]	60.5	5.1	60	53.4	70.1	0.9	1.8
Metals Dissolved							
Silver (Ag) [mg/L]	<0.0002	<0.0002	<0.0002	<0.0002	<0.005	<0.0002	<0.0002
Aluminium (Al) [mg/L]	0.95	0.12	0.09	0.09	0.09	0.06	0.02
Arsenic (As) [mg/L]	<0.0004	0.0007	<0.0004	0.0120	na	<0.0004	<0.0004
Boron (B) [mg/L]	0.074	0.088	0.641	0.684	<0.05	0.008	<0.002
Barium (Ba) [mg/L]	0.365	0.167	0.417	0.634	0.109	0.0015	0.0038
Beryllium (Be) [mg/L]	<0.0005	<0.0005	<0.0005	<0.0005	<0.001	<0.0005	<0.0005
Bismuth (Bi) [mg/L]	0.0002	<0.0001	<0.00005	<0.00005	na	<0.00005	0.00006
Cadmium (Cd) [mg/L]	<0.0001	<0.0001	0.0003	0.0003	<0.001	<0.0001	<0.0001
Cobalt (Co) [mg/L]	<0.0001	<0.0001	0.0012	0.0017	<0.002	<0.0001	0.0001
Chromium (Cr) [mg/L]	<0.0004	<0.0004	<0.0004	<0.0004	<0.005	<0.0004	<0.0004
Copper (Cu) [mg/L]	0.0144	0.0216	0.163	0.0235	<0.001	0.0063	0.0250
Iron (Fe) [mg/L]	<0.01	<0.01	<0.01	<0.01	<0.005	0.079	0.096
Manganese (Mn) [mg/L]	0.0045	0.0076	0.0035	0.0030	na	0.001	0.018
Molybdenum (Mo) [mg/L]	0.0068	0.0010	0.0307	0.0299	na	0.0001	0.0003
Nickel (Ni) [mg/L]	<0.0001	0.0012	0.0284	0.0096	0.003	<0.0001	0.0002
Phosphorus (P) [mg/L]	0.12	0.09	1.9	0.93	0.3	na	na
Lead (Pb) [mg/L]	0.0011	0.0016	0.0020	0.0006	<0.005	0.0006	0.0013
Antimony (Sb) [mg/L]	0.0040	0.0038	0.0064	0.0060	<0.05	0.0006	0.0005
Selenium (Se) [mg/L]	0.0015	0.0038	<0.0004	<0.0004	na	<0.0004	<0.0004
Tin (Sn) [mg/L]	0.0005	0.0004	<0.0002	<0.0002	na	<0.0002	<0.0002
Strontium (Sr) [mg/L]	3.14	0.406	1.97	1.51	0.520	0.0069	0.0082
Titanium (Ti) [mg/L]	<0.0003	<0.0003	<0.0003	<0.0003	<0.001	<0.0003	0.0007
Thallium (Tl) [mg/L]	<0.0000	<0.00005	<0.00005	<0.00005	<0.05	<0.00005	<0.00005
Uranium (U) [mg/L]	0.0043	<0.0001	0.0029	0.0173	na	<0.0001	<0.0001
Vanadium (V) [mg/L]	0.0011	0.0005	0.0031	0.0355	<0.001	<0.0001	<0.0001

(Continued)

Table A.1 (continued)

Water Quality Parameter	Water A	Water B	Water C	Water D	Water E	Water F	Water G
Zinc (Zn) [mg/L]	0.809	0.854	0.566	0.087	0.005	0.004	0.022
Bromide (mg/L)	na	Na	0.1	<0.1	<0.1	<0.1	<0.1
TOC (mg/L)	na	6.3*	1	6	<1	4	2
Colour (TCU)	na	NA	8	15	<3	30	20
Turbidity (NTU)	na	0.26	5.1*	47*	1.6*	0.15	0.49

na =Not available

All parameters measured by EnviroTest Laboratories Edmonton, Canada.

*Measured at the University of Alberta following Standard Methods.

APPENDIX B

RESULTS OF NEONATAL CD-1 INFECTIVITY ANALYIS FOR ALL EXPERIMENTAL TRIALS

Table B.1

Neonatal CD-1 infectivity analysis for experimental trials with natural water A

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
1	A	21	8	Ozone	1000	5	0	0		2.17	35
1	A	21	8	Ozone	10000	5	2	0.4	2.17		35
1	A	21	8	Ozone+Free Chlorine	10000	5	0	0		2.82	35
1	A	21	8	Ozone+Free Chlorine	100000	5	4	0.8	2.82		35
1	A	21	8	Free Chlorine	100	5	4	0.8	-0.18	-0.18	35
1	A	21	8	Free Chlorine	1000	5	5	1			35
1	A	21	8	Control	100	5	5	1		>-0.18	35
1	A	21	8	Control	1000	5	5	1			35
1	A	21	8	Control	100	5	3	0.6	0.01	0.01	35
1	A	21	8	Control	1000	5	5	1			35
2	A	21	8	Ozone	1000	5	2	0.4	1.17	1.17	35
2	A	21	8	Ozone	10000	5	5	1			35
2	A	21	8	Ozone+Free Chlorine	10000	5	4	0.8	1.82	1.82	35
2	A	21	8	Ozone+Free Chlorine	100000	5	5	1			35
2	A	21	8	Free Chlorine	100	5	1	0.2	0.36	0.36	35
2	A	21	8	Free Chlorine	1000	5	5	1			35
2	A	21	8	Control	100	5	2	0.4	0.17	0.17	35
2	A	21	8	Control	1000	5	5	1			35
2	A	21	8	Control	100	5	3	0.6	0.01	0.01	35
2	A	21	8	Control	1000	5	5	1			35
3	A	21	8	Ozone	1000	5	3	0.6	1.01	1.01	35
3	A	21	8	Ozone	10000	5	5	1			35
3	A	21	8	Ozone+Free Chlorine	1000	5	1	0.2	1.36	1.59	35
3	A	21	8	Ozone+Free Chlorine	10000	5	4	0.8	1.82		35
3	A	21	8	Free Chlorine	100	5	3	0.6	0.01	0.01	35

(Continued)

Table B.1 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
3	A	21	8	Free Chlorine	1000	5	5	1			35
3	A	21	8	Control	100	5	2	0.4	0.17	0.17	35
3	A	21	8	Control	100	5	4	0.8	-0.18	0.32	35
3	A	21	8	Control	1000	5	4	0.8	0.82		35
4	A	21	8	Ozone	1 000	5	0	0.00		2.17	35
4	A	21	8	Ozone	10 000	5	2	0.40	2.17		35
4	A	21	8	Ozone + Free Chlorine	1 000	5	0	0.00		1.82	35
4	A	21	8	Ozone + Free Chlorine	10 000	5	4	0.80	1.82		35
4	A	21	8	Chlorine	100	5	2	0.40	0.17	0.17	35
4	A	21	8	Chlorine	1 000	5	5	1.00			35
5	A	21	8	Ozone	100	5	2	0.40	0.17	0.17	35
5	A	21	8	Ozone	1 000	5	5	1.00			35
5	A	21	8	Ozone + Free Chlorine	1 000	4	3	0.75	0.87	0.87	35
5	A	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			35
5	A	21	8	Chlorine	100	5	3	0.60	0.01	0.01	35
5	A	21	8	Chlorine	1 000	5	5	1.00			35
5	A	21	8	Control	60	5	4	0.80	-0.40	-0.40	35
5	A	21	8	Control	600	5	5	1.00			35
5	A	21	8	Control	60	5	2	0.40	-0.06	-0.06	35
5	A	21	8	Control	600	5	5	1.00			35
6	A	21	8	Ozone	100	5	0	0.00		0.82	35
6	A	21	8	Ozone	1 000	5	4	0.80	0.82		35
6	A	21	8	Ozone + Free Chlorine	1 000	5	5	1.00		<0.82	35
6	A	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			35
6	A	21	8	Chlorine	100	4	1	0.25	0.30	0.30	35

(Continued)

Table B.1 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
6	A	21	8	Chlorine	1 000	5	5	1.00			35
6	A	21	8	Control	60	5	2	0.40	-0.06	0.27	35
6	A	21	8	Control	600	5	4	0.80	0.60		35
6	A	21	8	Control	60	5	5	1.00		<-0.40	35
6	A	21	8	Control	600	5	5	1.00			35
7	A	21	8	Ozone	1 000	5	1	0.20	1.36	1.68	35
7	A	21	8	Ozone	10 000	5	3	0.60	2.01		35
7	A	21	8	Ozone + Free Chlorine	10 000	5	2	0.40	2.17	2.17	35
7	A	21	8	Ozone + Free Chlorine	100 000	5	5	1.00			35
7	A	21	8	Chlorine	1 000	5	2	0.40	1.17	1.17	35
7	A	21	8	Chlorine	10 000	5	5	1.00			35
7	A	21	8	Control	50	4	1	0.25	0.00	0.00	35
7	A	21	8	Control	500	4	4	1.00			35
7	A	21	8	Control	50	5	4	0.80	-0.48	-0.48	35
7	A	21	8	Control	500	5	5	1.00			35
8	A	21	8	Ozone	1 000	5	1	0.20	1.36	1.76	35
8	A	21	8	Ozone	10 000	5	2	0.40	2.17		35
8	A	21	8	Ozone + Free Chlorine	10 000	4	1	0.25	2.30	2.30	35
8	A	21	8	Ozone + Free Chlorine	100 000	5	5	1.00			35
8	A	21	8	Chlorine	1 000	5	4	0.80	0.82	0.82	35
8	A	21	8	Chlorine	10 000	5	5	1.00			35
8	A	21	8	Control	50	5	2	0.40	-0.14	-0.14	35
8	A	21	8	Control	500	5	5	1.00			35
8	A	21	8	Control	50	5	1	0.20	0.05	0.05	35
8	A	21	8	Control	500	5	5	1.00			35

(Continued)

Table B.1 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
9	A	21	6	Ozone	1 000	5	0	0.00		2.01	35
9	A	21	6	Ozone	10 000	5	3	0.60	2.01		35
9	A	21	6	Ozone + Free Chlorine	10 000	5	0	0.00		>3.36	35
9	A	21	6	Ozone + Free Chlorine	100 000	5	0	0.00			35
9	A	21	6	Chlorine	1 000	5	4	0.80	0.82	0.82	35
9	A	21	6	Chlorine	10 000	5	5	1.00			35
9	A	21	6	Control	50	5	2	0.40	-0.14	0.19	35
9	A	21	6	Control	500	5	4	0.80	0.52		35
9	A	21	6	Control	50	5	5	1.00		0.52	35
9	A	21	6	Control	500	5	4	0.80	0.52		35
10	A	21	6	Ozone	1 000	5	0	0.00		2.36	35
10	A	21	6	Ozone	10 000	5	1	0.20	2.36		35
10	A	21	6	Ozone + Free Chlorine	10 000	5	0	0.00		3.36	35
10	A	21	6	Ozone + Free Chlorine	100 000	5	1	0.20	3.36		35
10	A	21	6	Chlorine	1 000	5	4	0.80	0.82	0.82	35
10	A	21	6	Chlorine	10 000	5	5	1.00			35
10	A	21	6	Control	50	5	5	1.00		1.05	35
10	A	21	6	Control	500	5	1	0.20	1.05		35
10	A	21	6	Control	50	5	2	0.40	-0.14	-0.14	35
10	A	21	6	Control	500	5	5	1.00			35
11	A	21	6	Ozone	100	5	0	0.00		0.82	35
11	A	21	6	Ozone	1 000	5	4	0.80	0.82		35
11	A	21	6	Ozone	10 000	5	5	1.00			35
11	A	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		2.36	35
11	A	21	6	Ozone + Free Chlorine	10 000	5	1	0.20	2.36		35
11	A	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			35

(Continued)

Table B.1 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
11	A	21	6	Chlorine	100	5	0	0.00		1.36	35
11	A	21	6	Chlorine	1 000	5	1	0.20	1.36		35
11	A	21	6	Control	50	5	2	0.40	-0.14	0.19	35
11	A	21	6	Control	500	5	4	0.80	0.52		35
12	A	21	6	Ozone	100	5	0	0.00		2.36	35
12	A	21	6	Ozone	1 000	5	0	0.00			35
12	A	21	6	Ozone	10 000	5	1	0.20	2.36		35
12	A	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		2.82	35
12	A	21	6	Ozone + Free Chlorine	10 000	5	0	0.00			35
12	A	21	6	Ozone + Free Chlorine	100 000	5	4	0.80	2.82		35
12	A	21	6	Chlorine	100	5	1	0.20	0.36	0.59	35
12	A	21	6	Chlorine	1 000	5	4	0.80	0.82		35
12	A	21	6	Control	50	5	0	0.00		0.71	35
12	A	21	6	Control	500	5	3	0.60	0.71		35
13	A	21	6	Ozone	100	5	0	0.00		1.32	35
13	A	21	6	Ozone	1 000	5	4	0.80	0.82		35
13	A	21	6	Ozone	10 000	5	4	0.80	1.82		35
13	A	21	6	Ozone + Free Chlorine	1 000	5	2	0.40	1.17	1.17	35
13	A	21	6	Ozone + Free Chlorine	10 000	5	5	1.00			35
13	A	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			35
13	A	21	6	Chlorine	100	5	0	0.00		0.82	35
13	A	21	6	Chlorine	1 000	5	4	0.80	0.82		35
13	A	21	6	Control	50	5	0	0.00		0.52	35
13	A	21	6	Control	500	5	4	0.80	0.52		35
14	A	21	6	Ozone	3 000	5	1	0.20	1.83	2.16	35

(Continued)

Table B.1 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
14	A	21	6	Ozone	30 000	5	3	0.60	2.49		35
14	A	21	6	Ozone + Free Chlorine	10 000	5	0	0.00		2.82	35
14	A	21	6	Ozone + Free Chlorine	100 000	5	4	0.80	2.82		35
14	A	21	6	Chlorine	100	5	2	0.40	0.17	0.49	35
14	A	21	6	Chlorine	1 000	5	4	0.80	0.82		35

Table B.2

Neonatal CD-1 infectivity analysis for experimental trials with natural water B

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
15	B	21	6	Ozone	1 000	5	4	0.80	0.81	0.81	36
15	B	21	6	Ozone	10 000	5	5	1.00			36
15	B	21	6	Ozone	100 000	5	5	1.00			36
15	B	21	6	Ozone + Free Chlorine	1 000	5	1	0.20	1.67	1.74	36
15	B	21	6	Ozone + Free Chlorine	10 000	5	4	0.80	1.81		36
15	B	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			36
15	B	21	6	Chlorine	100	5	3	0.60	0.11	0.11	36
15	B	21	6	Chlorine	1 000	5	5	1.00			36
15	B	21	6	Control	50	5	3	0.60	-0.19	-0.19	36
15	B	21	6	Control	500	5	5	1.00			36
16	B	21	6	Ozone	1 000	5	0	0.00		2.74	36
16	B	21	6	Ozone	10 000	5	1	0.20	2.67		36
16	B	21	6	Ozone	100 000	5	4	0.80	2.81		36
16	B	21	6	Ozone + Free Chlorine	1 000	5	5	1.00		< 0.81	36
16	B	21	6	Ozone + Free Chlorine	10 000	5	5	1.00			36
16	B	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			36
16	B	21	6	Chlorine	100	5	0	0.00		>1.67	36
16	B	21	6	Chlorine	1 000	5	1	0.20	1.67		36
16	B	21	6	Control	50	5	2	0.40	0.07	0.44	36
16	B	21	6	Control	500	5	3	0.60	0.81		36
17	B	21	6	Ozone	1 000	5	0	0.00		1.76	36
17	B	21	6	Ozone	10 000	5	5	1.00			36
17	B	21	6	Ozone	100 000	5	5	1.00			36
17	B	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		2.74	36
17	B	21	6	Ozone + Free Chlorine	10 000	5	1	0.20	2.67		36

(Continued)

Table B.2 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
17	B	21	6	Ozone + Free Chlorine	100 000	5	4	0.80	2.81		36
17	B	21	6	Chlorine	100	5	4	0.80	-0.19	-0.19	36
17	B	21	6	Chlorine	1 000	5	5	1.00			36
17	B	21	6	Control	50	5	2	0.40	0.07	0.07	36
17	B	21	6	Control	500	5	5	1.00			36
18	B	21	6	Ozone	1 000	5	5	1.00		< 0.81	36
18	B	21	6	Ozone	10 000	5	5	1.00			36
18	B	21	6	Ozone	100 000	5	5	1.00			36
18	B	21	6	Ozone + Free Chlorine	1 000	5	1	0.20	1.67	1.81	36
18	B	21	6	Ozone + Free Chlorine	10 000	5	4	0.80	1.81		36
18	B	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			36
18	B	21	6	Chlorine	100	5	4	0.80	-0.19	-0.19	36
18	B	21	6	Chlorine	1 000	5	5	1.00			36
18	B	21	6	Control	50	5	2	0.40	0.07	0.29	36
18	B	21	6	Control	500	5	4	0.80	0.51		36
19	B	21	6	Ozone	1 000	5	0	0.00		2.81	36
19	B	21	6	Ozone	10 000	5	0	0.00			36
19	B	21	6	Ozone	100 000	5	4	0.80	2.81		36
19	B	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		3.20	36
19	B	21	6	Ozone + Free Chlorine	10 000	5	0	0.00			36
19	B	21	6	Ozone + Free Chlorine	100 000	5	3	0.60	3.11		36
19	B	21	6	Chlorine	50	5	0	0.00		0.51	36
19	B	21	6	Chlorine	500	5	4	0.80	0.51		36
19	B	21	6	Control	50	5	2	0.40	0.07	0.07	36
19	B	21	6	Control	500	5	5	1.00			36

(Continued)

Table B.2 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
20	B	21	6	Ozone	1 000	5	0	0.00		2.74	36
20	B	21	6	Ozone	10 000	5	1	0.20	2.67		36
20	B	21	6	Ozone	100 000	5	4	0.80	2.81		36
20	B	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		2.81	36
20	B	21	6	Ozone + Free Chlorine	10 000	5	0	0.00			36
20	B	21	6	Ozone + Free Chlorine	100 000	5	4	0.80	2.81		36
20	B	21	6	Chlorine	50	5	4	0.80	-0.49	-0.49	36
20	B	21	6	Chlorine	500	5	5	1.00			36
20	B	21	6	Control	50	5	2	0.40	0.07	0.44	36
20	B	21	6	Control	500	5	3	0.60	0.81		36
21	B	21	6	Ozone	1 000	5	0	0.00		3.02	36
21	B	21	6	Ozone	10 000	5	1	0.20	2.67		36
21	B	21	6	Ozone	100 000	5	2	0.40	3.37		36
21	B	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		3.37	36
21	B	21	6	Ozone + Free Chlorine	10 000	5	0	0.00			36
21	B	21	6	Ozone + Free Chlorine	100 000	5	2	0.40	3.37		36
21	B	21	6	Chlorine	50	5	2	0.40	0.07	0.07	36
21	B	21	6	Chlorine	500	5	5	1.00			36
22	B	21	6	Ozone	100	5	1	0.20	0.67	0.74	36
22	B	21	6	Ozone	1 000	5	4	0.80	0.81		36
22	B	21	6	Ozone	10 000	5	5	1.00			36
22	B	21	6	Ozone + Free Chlorine	100	5	0	0.00		1.11	36
22	B	21	6	Ozone + Free Chlorine	1 000	5	3	0.60	1.11		36
22	B	21	6	Ozone + Free Chlorine	10 000	5	5	1.00			36
22	B	21	6	Chlorine	50	5	2	0.40	0.07	0.07	36
22	B	21	6	Chlorine	500	5	5	1.00			36

(Continued)

Table B.2 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
22	B	21	6	Control	50	5	3	0.60	-0.19	-0.19	36
22	B	21	6	Control	500	5	5	1.00			36
23	B	21	6	Ozone	100	5	1	0.20	0.67	0.67	36
23	B	21	6	Ozone	1 000	5	5	1.00			36
23	B	21	6	Ozone	10 000	5	5	1.00			36
23	B	21	6	Ozone + Free Chlorine	1 000	5	1	0.20	1.67	2.24	36
23	B	21	6	Ozone + Free Chlorine	10 000	5	0	0.00			36
23	B	21	6	Ozone + Free Chlorine	100 000	5	4	0.80	2.81		36
23	B	21	6	Chlorine	50	5	0	0.00		1.07	36
23	B	21	6	Chlorine	500	5	2	0.40	1.07		36
23	B	21	6	Control	50	5	1	0.20	0.37	0.37	36
23	B	21	6	Control	500	5	4	0.80	0.51		36
24	B	21	6	Ozone	100	5	1	0.20	0.67	0.74	36
24	B	21	6	Ozone	1 000	5	4	0.80	0.81		36
24	B	21	6	Ozone	10 000	5	5	1.00			36
24	B	21	6	Ozone + Free Chlorine	1 000	5	4	0.80	0.81	0.81	36
24	B	21	6	Ozone + Free Chlorine	10 000	5	5	1.00			36
24	B	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			36
24	B	21	6	Chlorine	50	5	3	0.60	-0.19	0.31	36
24	B	21	6	Chlorine	500	5	3	0.60	0.81		36
24	B	21	6	Control	50	5	1	0.20	0.37	0.37	36
24	B	21	6	Control	500	5	5	1.00			36
25	B	21	6	Ozone	100	5	1	0.20	0.67	0.89	36
25	B	21	6	Ozone	1 000	5	3	0.60	1.11		36
25	B	21	6	Ozone	10 000	5	5	1.00			36

(Continued)

Table B.2 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
25	B	21	6	Ozone + Free Chlorine	1 000	5	4	0.80	0.81	1.31	36
25	B	21	6	Ozone + Free Chlorine	10 000	5	4	0.80	1.81		36
25	B	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			36
25	B	21	6	Chlorine	50	5	1	0.20	0.37	0.37	36
25	B	21	6	Chlorine	500	5	5	1.00			36
25	B	21	6	Control	50	5	3	0.60	-0.19	-0.19	36
25	B	21	6	Control	500	5	5	1.00			36
26	B	21	6	Ozone	100	5	1	0.20	0.67	0.89	36
26	B	21	6	Ozone	1 000	5	3	0.60	1.11		36
26	B	21	6	Ozone	10 000	5	5	1.00			36
26	B	21	6	Ozone + Free Chlorine	1 000	5	1	0.20	1.67	1.89	36
26	B	21	6	Ozone + Free Chlorine	10 000	5	3	0.60	2.11		36
26	B	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			36
26	B	21	6	Chlorine	50	5	2	0.40	0.07	0.07	36
26	B	21	6	Chlorine	500	5	5	1.00			36
26	B	21	6	Control	50	5	1	0.20	0.37	0.44	36
26	B	21	6	Control	500	5	4	0.80	0.51		36
27	B	1	6	Ozone	200	5	0	0.00		2.44	37
27	B	1	6	Ozone	2 000	5	0	0.00			37
27	B	1	6	Ozone	20 000	5	3	0.60	2.44		37
27	B	1	6	Ozone + Free Chlorine	200	5	0	0.00		>2.81	37
27	B	1	6	Ozone + Free Chlorine	2 000	5	0	0.00			37
27	B	1	6	Ozone + Free Chlorine	20 000	5	0	0.00			37
27	B	1	6	Chlorine	50	5	0	0.00		0.63	37
27	B	1	6	Chlorine	500	5	4	0.80	0.63		37
27	B	1	6	Control	50	5	2	0.40	0.01	0.01	37

(Continued)

Table B.2 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
27	B	1	6	Control	500	5	5	1.00			37
28	B	1	6	Ozone	200	5	0	0.00		2.02	37
28	B	1	6	Ozone	2 000	5	1	0.20	1.81		37
28	B	1	6	Ozone	20 000	5	4	0.80	2.23		37
28	B	1	6	Ozone + Free Chlorine	200	5	0	0.00		2.44	37
28	B	1	6	Ozone + Free Chlorine	2 000	5	0	0.00			37
28	B	1	6	Ozone + Free Chlorine	20 000	5	3	0.60	2.44		37
28	B	1	6	Chlorine	50	5	3	0.60	-0.16	0.23	37
28	B	1	6	Chlorine	500	5	4	0.80	0.63		37
28	B	1	6	Control	50	5	1	0.20	0.21	0.21	37
28	B	1	6	Control	500	5	5	1.00			37
29	B	1	6	Ozone	100	5	2	0.40	0.31	0.31	37
29	B	1	6	Ozone	1 000	5	5	1.00			37
29	B	1	6	Ozone	10 000	5	5	1.00			37
29	B	1	6	Ozone + Free Chlorine	100	5	2	0.40	0.31	0.31	37
29	B	1	6	Ozone + Free Chlorine	1 000	5	5	1.00			37
29	B	1	6	Ozone + Free Chlorine	10 000	5	5	1.00			37
29	B	1	6	Chlorine	50	5	2	0.40	0.01	0.01	37
29	B	1	6	Chlorine	500	5	5	1.00			37
29	B	1	6	Control	50	5	4	0.80	-0.37	0.13	37
29	B	1	6	Control	500	5	4	0.80	0.63		37
30	B	1	6	Ozone	500	5	5	1.00		<0.63	37
30	B	1	6	Ozone	5 000	5	5	1.00			37
30	B	1	6	Ozone	50 000	5	5	1.00			37
30	B	1	6	Ozone + Free Chlorine	500	5	5	1.00		<0.63	37

(Continued)

Table B.2 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
30	B	1	6	Ozone + Free Chlorine	5 000	5	5	1.00			37
30	B	1	6	Ozone + Free Chlorine	50 000	5	5	1.00			37
30	B	1	6	Chlorine	50	5	2	0.40	0.01	0.01	37
30	B	1	6	Chlorine	500	5	5	1.00			37
30	B	1	6	Control	50	5	1	0.20	0.21	0.42	37
30	B	1	6	Control	500	5	4	0.80	0.63		37
31	B	1	6	Ozone	1 000	5	0	0.00		2.22	37
31	B	1	6	Ozone	10 000	5	5	1.00			37
31	B	1	6	Ozone	100 000	5	5	1.00			37
31	B	1	6	Ozone + Free Chlorine	1 000	5	0	0.00		2.51	37
31	B	1	6	Ozone + Free Chlorine	10 000	5	1	0.20	2.51		37
31	B	1	6	Ozone + Free Chlorine	100 000	5	5	1.00			37
31	B	1	6	Chlorine	50	5	1	0.20	0.21	0.21	37
31	B	1	6	Chlorine	500	5	5	1.00			37
31	B	1	6	Control	50	5	2	0.40	0.01	0.42	37
31	B	1	6	Control	500	5	3	0.60	0.84		37
32	B	1	6	Ozone	100	5	3	0.60	0.14	0.14	37
32	B	1	6	Ozone	1 000	5	5	1.00			37
32	B	1	6	Ozone	10 000	5	5	1.00			37
32	B	1	6	Ozone + Free Chlorine	100	5	3	0.60	0.14	0.14	37
32	B	1	6	Ozone + Free Chlorine	1 000	5	5	1.00			37
32	B	1	6	Ozone + Free Chlorine	10 000	5	5	1.00			37
32	B	1	6	Chlorine	50	5	1	0.20	0.21	0.21	37
32	B	1	6	Chlorine	500	5	5	1.00			37
32	B	1	6	Control	50	5	2	0.40	0.01	0.01	37
32	B	1	6	Control	500	5	5	1.00			37

(Continued)

Table B.2 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
33	B	1	6	Ozone	50	5	2	0.40	0.01	0.01	37
33	B	1	6	Ozone	500	5	5	1.00			37
33	B	1	6	Ozone	5 000	5	5	1.00			37
33	B	1	6	Ozone + Free Chlorine	50	5	0	0.00		0.92	37
33	B	1	6	Ozone + Free Chlorine	500	5	5	1.00			37
33	B	1	6	Ozone + Free Chlorine	5 000	5	5	1.00			37
33	B	1	6	Chlorine	50	5	2	0.40	0.01	0.01	37
33	B	1	6	Chlorine	500	5	5	1.00			37
33	B	1	6	Control	50	5	0	0.00		0.63	37
33	B	1	6	Control	500	5	4	0.80	0.63		37
34	B	1	6	Ozone	500	5	0	0.00		2.42	37
34	B	1	6	Ozone	5 000	5	2	0.40	2.01		37
34	B	1	6	Ozone	50 000	5	3	0.60	2.84		37
34	B	1	6	Ozone + Free Chlorine	1 000	5	0	0.00		2.31	37
34	B	1	6	Ozone + Free Chlorine	10 000	5	2	0.40	2.31		37
34	B	1	6	Ozone + Free Chlorine	100 000	5	1(CENSORED)				37
34	B	1	6	Chlorine	50	5	2	0.40	0.01	0.32	37
34	B	1	6	Chlorine	500	5	4	0.80	0.63		37
34	B	1	6	Control	50	5	0	0.00		0.63	37
34	B	1	6	Control	500	5	4	0.80	0.63		37
35	B	1	6	Ozone	500	5	1	0.20	1.21	1.42	37
35	B	1	6	Ozone	5 000	5	4	0.80	1.63		37
35	B	1	6	Ozone	50 000	5	5	1.00			37
35	B	1	6	Ozone + Free Chlorine	500	5	0	0.00		2.21	37
35	B	1	6	Ozone + Free Chlorine	5 000	5	1	0.20	2.21		37

(Continued)

Table B.2 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
35	B	1	6	Ozone + Free Chlorine	50 000	5	5	1.00			37
35	B	1	6	Chlorine	50	5	3	0.60	-0.16	-0.16	37
35	B	1	6	Chlorine	500	5	5	1.00			37
35	B	1	6	Control	50	5	1	0.20	0.21	0.21	37
35	B	1	6	Control	500	5	5	1.00			37
36	B	1	6	Ozone	500	5	0	0.00		2.42	37
36	B	1	6	Ozone	5 000	5	0	0.00			37
36	B	1	6	Ozone	50 000	5	5	1.00			37
36	B	1	6	Ozone + Free Chlorine	1 000	5	0	0.00		3.01	37
36	B	1	6	Ozone + Free Chlorine	10 000	5	1	0.20	2.51		37
36	B	1	6	Ozone + Free Chlorine	100 000	5	1	0.20	3.51		37
36	B	1	6	Chlorine	50	5	3	0.60	-0.16	-0.16	37
36	B	1	6	Chlorine	500	5	5	1.00			37
36	B	1	6	Control	50	5	1	0.20	0.21	0.21	37
36	B	1	6	Control	500	5	5	1.00			37

Table B.3

Neonatal CD-1 infectivity analysis for experimental trials with natural water C

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
37	C	21	6	Ozone	500	5	0	0.00		>3.34	38
37	C	21	6	Ozone	5 000	5	0	0.00			38
37	C	21	6	Ozone	50 000	5	0	0.00			38
37	C	21	6	Ozone + Free Chlorine	500	5	0	0.00		>3.64	38
37	C	21	6	Ozone + Free Chlorine	5 000	5	0	0.00			38
37	C	21	6	Ozone + Free Chlorine	50 000	5	0	0.00			38
37	C	21	6	Chlorine	50	5	0	0.00		0.47	38
37	C	21	6	Chlorine	500	5	4	0.80	0.47		38
37	C	21	6	Control	50	5	1	0.20	0.47	0.47	38
37	C	21	6	Control	500	5	5	1.00			38
38	C	21	6	Ozone	500	5	0	0.00		>3.34	38
38	C	21	6	Ozone	5 000	5	0	0.00			38
38	C	21	6	Ozone	50 000	5	0	0.00			38
38	C	21	6	Ozone + Free Chlorine	500	5	0	0.00		>3.34	38
38	C	21	6	Ozone + Free Chlorine	5 000	5	0	0.00			38
38	C	21	6	Ozone + Free Chlorine	50 000	5	0	0.00			38
38	C	21	6	Chlorine	100	5	1	0.20	0.77	0.77	38
38	C	21	6	Chlorine	1 000	5	5	1.00			38
38	C	21	6	Control	50	5	2	0.40	0.12	0.12	38
38	C	21	6	Control	500	5	5	1.00			38
39	C	21	6	Ozone	500	5	0	0.00		>3.34	38
39	C	21	6	Ozone	5 000	5	0	0.00			38
39	C	21	6	Ozone	50 000	5	0	0.00			38
39	C	21	6	Ozone + Free Chlorine	500	5	0	0.00		>3.34	38
39	C	21	6	Ozone + Free Chlorine	5 000	5	0	0.00			38

(Continued)

Table B.3 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
39	C	21	6	Ozone + Free Chlorine	50 000	5	0	0.00			38
39	C	21	6	Chlorine	50	5	1	0.20	0.47	0.47	38
39	C	21	6	Chlorine	500	5	5	1.00			38
39	C	21	6	Control	50	5	2	0.40	0.12	0.12	38
39	C	21	6	Control	500	5	5	1.00			38
40	C	21	6	Ozone	1 000	5	0	0.00		2.45	38
40	C	21	6	Ozone	10 000	5	3	0.60	2.13		38
40	C	21	6	Ozone	100 000	5	4	0.80	2.77		38
40	C	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		3.77	38
40	C	21	6	Ozone + Free Chlorine	10 000	5	0	0.00			38
40	C	21	6	Ozone + Free Chlorine	100 000	5	1	0.20	3.77		38
40	C	21	6	Ozone	1 000	5	3	0.60	1.13	1.13	38
40	C	21	6	Ozone	10 000	5	5	1.00			38
40	C	21	6	Ozone	100 000	5	5	1.00			38
40	C	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		2.13	38
40	C	21	6	Ozone + Free Chlorine	10 000	5	3	0.60	2.13		38
40	C	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			38
40	C	21	6	Control	50	5	3	0.60	-0.17	-0.17	38
40	C	21	6	Control	500	5	5	1.00			38
41	C	21	6	Ozone	1 000	5	0	0.00		2.42	38
41	C	21	6	Ozone	10 000	5	2	0.40	2.42		38
41	C	21	6	Ozone	100 000	5	5	1.00			38
41	C	21	6	Ozone + Free Chlorine	1 000	5	2	0.40	1.42	1.42	38
41	C	21	6	Ozone + Free Chlorine	10 000	5	5	1.00			38
41	C	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			38
41	C	21	6	Ozone	1 000	5	5	1.00		<1.01	38

(Continued)

Table B.3 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
41	C	21	6	Ozone	10 000	5	5	1.00			38
41	C	21	6	Ozone	100 000	5	5	1.00			38
41	C	21	6	Ozone + Free Chlorine	1 000	5	2	0.40	1.42	1.42	38
41	C	21	6	Ozone + Free Chlorine	10 000	5	5	1.00			38
41	C	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			38
41	C	21	6	Control	50	5	4	0.80	-0.53	-0.53	38
41	C	21	6	Control	500	5	5	1.00			38
42	C	21	8	Ozone	1 000	5	2	0.40	1.42	1.77	38
42	C	21	8	Ozone	10 000	5	3	0.60	2.13		38
42	C	21	8	Ozone	100 000	5	5	1.00			38
42	C	21	8	Ozone + Free Chlorine	1 000	5	0	0.00		2.13	38
42	C	21	8	Ozone + Free Chlorine	10 000	5	3	0.60	2.13		38
42	C	21	8	Ozone + Free Chlorine	100 000	5	5	1.00			38
42	C	21	8	Ozone + Free Chlorine	1 000	5	0	0.00		2.79	38
42	C	21	8	Ozone + Free Chlorine	10 000	5	0	0.00			38
42	C	21	8	Ozone + Free Chlorine	100 000	5	5	1.00			38
42	C	21	8	Chlorine	50	5	1	0.20	0.47	0.47	38
42	C	21	8	Chlorine	500	5	5	1.00			38
42	C	21	8	Chlorine	50	5	2	0.40	0.12	0.12	38
42	C	21	8	Chlorine	500	5	5	1.00			38
42	C	21	8	Control	50	5	2	0.40	0.12	0.12	38
42	C	21	8	Control	500	5	5	1.00			38
43	C	21	8	Ozone	1 000	5	5	1.00		<1.01	38
43	C	21	8	Ozone	10 000	5	5	1.00			38
43	C	21	8	Ozone	100 000	5	5	1.00			38
43	C	21	8	Ozone + Free Chlorine	1 000	5	5	1.00		<1.01	38

(Continued)

Table B.3 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
43	C	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			38
43	C	21	8	Ozone + Free Chlorine	100 000	5	5	1.00			38
43	C	21	8	Ozone + Free Chlorine	1 000	5	5	1.00		<1.01	38
43	C	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			38
43	C	21	8	Ozone + Free Chlorine	100 000	5	5	1.00			38
43	C	21	8	Chlorine	50	5	1	0.20	0.47	0.47	38
43	C	21	8	Chlorine	500	4	4	1.00			38
43	C	21	8	Chlorine	50	5	1	0.20	0.47	0.47	38
43	C	21	8	Chlorine	500	5	4	0.80	0.47		38
43	C	21	8	Control	50	5	2	0.40	0.12	0.12	38
43	C	21	8	Control	500	None	None				38
44	C	21	8	Ozone	1 000	5	5	1.00		<0.74	38
44	C	21	8	Ozone	10 000	5	5	1.00			38
44	C	21	8	Ozone	100 000	5	not done				38
44	C	21	8	Ozone + Free Chlorine	1 000	2	2	1.00		<1.24	38
44	C	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			38
44	C	21	8	Ozone + Free Chlorine	100 000	5	5	1.00			38
44	C	1	6	Ozone + Free Chlorine	1 000	5	0	0.00			38
44	C	1	8	Ozone + Free Chlorine	10 000	5	0	0.00			38
44	C	1	8	Ozone + Free Chlorine	100 000	5	0	0.00			38
44	C	1	8	Chlorine	50	5	0	0.00			38
44	C	1	8	Chlorine	500	5	0	0.00			38
44	C	21	8	Chlorine	50	5	1	0.20	0.47	0.47	38
44	C	21	8	Chlorine	500	5	5	1.00			38
44	C	21	8	Control	50	5	3	0.60	-0.17	-0.17	38
44	C	21	8	Control	500	5	5	1.00			38

(Continued)

Table B.3 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
45	C	21	6	Ozone	1 000	5	1	0.20	1.77	1.77	38
45	C	21	6	Ozone	10 000	5	4	0.80	1.77		38
45	C	21	6	Ozone	100 000	5	5	1.00			38
45	C	21	6	Ozone + Free Chlorine	1 000	5	1	0.20	1.77	1.95	38
45	C	21	6	Ozone + Free Chlorine	10 000	5	3	0.60	2.13		38
45	C	21	6	Ozone + Free Chlorine	100 000	5	5	1.00			38
45	C	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		>3.77	38
45	C	21	6	Ozone + Free Chlorine	10 000	5	0	0.00			38
45	C	21	6	Ozone + Free Chlorine	100 000	5	0	0.00			38
45	C	21	6	Chlorine	50	5	4	0.80	-0.53	-0.53	38
45	C	21	6	Chlorine	500	5	5	1.00			38
45	C	21	6	Chlorine	50	5	2	0.40	0.12	0.47	38
45	C	21	6	Chlorine	500	5	3	0.60	0.83		38
45	C	21	6	Control	50	5	1	0.20	0.47	0.47	38
45	C	21	6	Control	500	5	5	1.00			38
46	C	21	8	Ozone	100	5	0	0.00		1.42	38
46	C	21	8	Ozone	1 000	5	2	0.40	1.42		38
46	C	21	8	Ozone	10 000	5	5	1.00			38
46	C	21	8	Ozone + Free Chlorine	500	5	1	0.20	1.47	1.47	38
46	C	21	8	Ozone + Free Chlorine	5 000	5	4	0.80	1.47		38
46	C	21	8	Ozone + Free Chlorine	50 000	5	5	1.00			38
46	C	21	8	Ozone + Free Chlorine	1 000	5	0	0.00		2.42	38
46	C	21	8	Ozone + Free Chlorine	10 000	5	2	0.40	2.42		38
46	C	21	8	Ozone + Free Chlorine	100 000	5	5	1.00			38
46	C	21	8	Chlorine	50	5	2	0.40	0.12	0.30	38
46	C	21	8	Chlorine	500	5	4	0.80	0.47		38
46	C	21	8	Chlorine	50	5	1	0.20	0.47	0.47	38

(Continued)

Table B.3 (Continued)

46	C	21	8	Chlorine	500	5	5	1.00			38
46	C	21	8	Control	50	5	1	0.20	0.47	0.47	38
46	C	21	8	Control	500	5	5	1.00			38
47	C	21	6	Ozone	100	5	3	0.60	0.13	0.13	38
47	C	21	6	Ozone	1 000	5	5	1.00			38
47	C	21	6	Ozone	10 000	5	5	1.00			38
47	C	21	6	Ozone + Free Chlorine	100	5	1	0.20	0.77	0.77	38
47	C	21	6	Ozone + Free Chlorine	1 000	5	4	0.80	0.77		38
47	C	21	6	Ozone + Free Chlorine	10 000	5	5	1.00			38
47	C	21	6	Ozone + Free Chlorine	100	5	3	0.60	Sample mixup	1.77	38
47	C	21	6	Ozone + Free Chlorine	1 000	5	5	1.00	Sample mixup		38
47	C	21	6	Ozone + Free Chlorine	10 000	5	4	0.80	1.77		38
47	C	21	6	Control	50	5	0	0.00			38
47	C	21	6	Control	500	5	0	0.00			38
48	C	21	8	Ozone	100	5	3	0.60	0.13	0.13	38
48	C	21	8	Ozone	1 000	5	5	1.00			38
48	C	21	8	Ozone	10 000	5	5	1.00			38
48	C	21	8	Ozone + Free Chlorine	100	5	1	0.20	0.77	1.11	38
48	C	21	8	Ozone + Free Chlorine	1 000	5	4	0.80	0.77		38
48	C	21	8	Ozone + Free Chlorine	10 000	5	4	0.80	1.77		38
48	C	21	8	Ozone + Free Chlorine	100	5	1	0.20	0.77	0.77	38
48	C	21	8	Ozone + Free Chlorine	1 000	5	4	0.80	0.77		38
48	C	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			38
48	C	21	8	Chlorine	50	5	2	0.40	0.12	0.30	38
48	C	21	8	Chlorine	500	5	4	0.80	0.47		38
48	C	21	8	Chlorine	50	5	2	0.40	0.12	0.12	38
48	C	21	8	Chlorine	500	5	5	1.00			38

(Continued)

Table B.3 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
48	C	21	8	Control	50	5	2	0.40	0.12	0.12	38
48	C	21	8	Control	500	5	5	1.00			38
49	C	21	6	Ozone	100	5	0	0.00		1.77	38
49	C	21	6	Ozone	1 000	5	1	0.20	1.77		38
49	C	21	6	Ozone	10 000	5	4	0.80	1.77		38
49	C	21	6	Ozone + Free Chlorine	500	5	1	0.20	1.47	1.97	38
49	C	21	6	Ozone + Free Chlorine	5 000	5	1	0.20	2.47		38
49	C	21	6	Ozone + Free Chlorine	50 000	5	5	1.00			38
49	C	21	6	Ozone + Free Chlorine	1 000	5	0	0.00		3.13	38
49	C	21	6	Ozone + Free Chlorine	10 000	5	0	0.00			38
49	C	21	6	Ozone + Free Chlorine	100 000	5	3	0.60	3.13		38
49	C	21	6	Chlorine	50	5	5	1.00		<-0.53	38
49	C	21	6	Chlorine	500	5	5	1.00			38
49	C	21	6	Chlorine	50	5	0	0.00		0.83	38
49	C	21	6	Chlorine	500	5	3	0.60	0.83		38
49	C	21	6	Control	50	5	1	0.20	0.47	0.47	38
50	C	21	6	Ozone	100	5	1	0.20	0.77	0.77	38
50	C	21	6	Ozone	1 000	5	4	0.80	0.77		38
50	C	21	6	Ozone	10 000	5	5	1.00			38
50	C	21	6	Ozone + Free Chlorine	100	5	1	0.20	0.77	0.77	38
50	C	21	6	Ozone + Free Chlorine	1 000	5	5	1.00			38
50	C	21	6	Ozone + Free Chlorine	10 000	5	5	1.00			38
50	C	21	6	Ozone + Free Chlorine	500	5	0	0.00		2.47	38
50	C	21	6	Ozone + Free Chlorine	5 000	5	1	0.20	2.47		38
50	C	21	6	Ozone + Free Chlorine	50 000	5	4	0.80	2.47		38
50	C	21	6	Chlorine	50	5	2	0.40	0.12	0.62	38

(Continued)

Table B.3 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
50	C	21	6	Chlorine	500	5	2	0.40	1.12		38
50	C	21	6	Chlorine	50	5	0	0.00		1.47	38
50	C	21	6	Chlorine	500	5	1	0.20	1.47		38
50	C	21	6	Control	50	5	3	0.60	-0.17	0.15	38
50	C	21	6	Control	500	5	4	0.80	0.47		38
51	C	21	8	Ozone	100	5	0	0.00		1.40	39
51	C	21	8	Ozone	1 000	5	1	0.20	1.57		39
51	C	21	8	Ozone	10 000	5	4	0.80	1.23		39
51	C	21	8	Ozone + Free Chlorine	100	5	0	0.00		2.57	39
51	C	21	8	Ozone + Free Chlorine	1 000	5	0	0.00			39
51	C	21	8	Ozone + Free Chlorine	10 000	5	1	0.20	2.57		39
52	C	21	8	Ozone	1 000	5	3	0.60	0.70	0.96	39
52	C	21	8	Ozone	10 000	5	4	0.80	1.23		39
52	C	21	8	Ozone	100 000	5	5	1.00			39
52	C	21	8	Ozone + Free Chlorine	100	5	1	0.20	0.57	1.07	39
52	C	21	8	Ozone + Free Chlorine	1 000	5	1	0.20	1.57		39
52	C	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			39
52	C	21	8	Ozone + Free Chlorine	100	5	0	0.00		1.40	39
52	C	21	8	Ozone + Free Chlorine	1 000	5	1	0.20	1.57		39
52	C	21	8	Ozone + Free Chlorine	10 000	5	4	0.80	1.23		39
52	C	21	8	Control	50	5	2	0.40	-0.21	0.10	39
52	C	21	8	Control	500	5	3	0.60	0.40		39

Table B.4

Neonatal CD-1 infectivity analysis for experimental trials with natural water D

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
52	C	21	8	Ozone	1 000	5	3	0.60	0.70	0.96	39
52	C	21	8	Ozone	10 000	5	4	0.80	1.23		39
52	C	21	8	Ozone	100 000	5	5	1.00			39
52	C	21	8	Ozone + Free Chlorine	100	5	1	0.20	0.57	1.07	39
52	C	21	8	Ozone + Free Chlorine	1 000	5	1	0.20	1.57		39
52	C	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			39
52	C	21	8	Ozone + Free Chlorine	100	5	0	0.00		1.40	39
52	C	21	8	Ozone + Free Chlorine	1 000	5	1	0.20	1.57		39
52	C	21	8	Ozone + Free Chlorine	10 000	5	4	0.80	1.23		39
52	C	21	8	Control	50	5	2	0.40	-0.21	0.10	39
52	C	21	8	Control	500	5	3	0.60	0.40		39
53	D	21	8	Ozone	100	5	3	0.60	-0.30	-0.30	39
53	D	21	8	Ozone	1 000	5	5	1.00			39
53	D	21	8	Ozone	10 000	5	5	1.00			39
53	D	21	8	Ozone + Free Chlorine	100	5	0	0.00		0.23	39
53	D	21	8	Ozone + Free Chlorine	1 000	5	4	0.80	0.23		39
53	D	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			39
53	D	21	8	Ozone + Free Chlorine	100	5	0	0.00		1.09	39
53	D	21	8	Ozone + Free Chlorine	1 000	5	2	0.40	1.09		39
53	D	21	8	Ozone + Free Chlorine	10 000	5	5	1.00			39
53	D	21	8	Ozone + Free Chlorine	1 000	5	0	0.00		1.36	39
53	D	21	8	Ozone + Free Chlorine	10 000	4	3	0.75	1.36		39
53	D	21	8	Ozone + Free Chlorine	100 000	3	3	1.00			39
53	D	21	8	Control	50	5	1	0.20	0.27	0.27	39
53	D	21	8	Control	500	5	5	1.00			39

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
55	D	21	8	Ozone	100	5	0	0		1.83	39
55	D	21	8	Ozone	1000	5	1	0.2	1.57		39
55	D	21	8	Ozone	10000	5	2	0.4	2.09		39
55	D	21	8	Ozone + Free Chlorine	500	5	0	0		1.40	39
55	D	21	8	Ozone + Free Chlorine	5000	5	3	0.6	1.40		39
55	D	21	8	Ozone + Free Chlorine	50000	5	5	1			39
55	D	21	8	Ozone + Free Chlorine	2000	5	1	0.2	1.87	1.94	39
55	D	21	8	Ozone + Free Chlorine	20000	5	3	0.6	2.00		39
55	D	21	8	Ozone + Free Chlorine	200000	5	5	1			39
55	D	21	8	Chlorine	50	5	1	0.2	0.27	0.34	39
55	D	21	8	Chlorine	500	5	3	0.6	0.40		39
55	D	21	8	Chlorine	50	5	1	0.2	0.27	0.27	39
55	D	21	8	Chlorine	500	5	5	1			39
55	D	21	8	Control	50	5	0	0			39
56	D	21	8	Ozone	100	5	0	0		2.57	39
56	D	21	8	Ozone	1000	5	0	0			39
56	D	21	8	Ozone	10000	5	1	0.2	2.57		39
56	D	21	8	Ozone + Free Chlorine	500	5	0	0		2.40	39
56	D	21	8	Ozone + Free Chlorine	5000	5	1	0.2	2.27		39
56	D	21	8	Ozone + Free Chlorine	50000	5	3	0.6	2.40		39
56	D	21	8	Ozone + Free Chlorine	2000	5	0	0		2.58	39
56	D	21	8	Ozone + Free Chlorine	20000	5	0	0			39
56	D	21	8	Ozone + Free Chlorine	200000	5	5	1			39
56	D	21	8	Chlorine	50	5	3	0.6	-0.60	-0.60	39
56	D	21	8	Chlorine	500	5	5	1			39
56	D	21	8	Chlorine	50	5	1	0.2	0.27	0.10	39
56	D	21	8	Chlorine	500	5	4	0.8	-0.08		39

(Continued)

Table B.4 (Continued)

56	D	21	8	Control	50	5	2	0.4	-0.21	-0.21	39
57	D	21	8	Ozone	100	4	0	0		2.57	39
57	D	21	8	Ozone	1000	5	0	0			39
57	D	21	8	Ozone	10000	5	1	0.2	2.57		39
57	D	21	8	Ozone + Free Chlorine	500	5	0	0		2.34	39
57	D	21	8	Ozone + Free Chlorine	5000	5	1	0.2	2.27		39
57	D	21	8	Ozone + Free Chlorine	50000	5	3	0.6	2.40		39
57	D	21	8	Ozone + Free Chlorine	2000	5	0	0		2.94	39
57	D	21	8	Ozone + Free Chlorine	20000	5	1	0.2	2.87		39
57	D	21	8	Ozone + Free Chlorine	200000	5	3	0.6	3.00		39
57	D	21	8	Chlorine	50	5	2	0.4	-0.21	-0.21	39
57	D	21	8	Chlorine	500	5	5	1			39
57	D	21	8	Chlorine	50	5	2	0.4	-0.21	0.10	39
57	D	21	8	Chlorine	500	5	3	0.6	0.40		39
57	D	21	8	Control	50	5	3	0.6	-0.60	-0.60	39
57	D	21	8	Control	500	5	5	1			39
58	D	21	6	Ozone	100	5	0	0		>2.57	39
58	D	21	6	Ozone	1000	5	0	0			39
58	D	21	6	Ozone	10000	5	0	0			39
58	D	21	6	Ozone + Free Chlorine	500	5	0	0		>3.27	39
58	D	21	6	Ozone + Free Chlorine	5000	5	0	0			39
58	D	21	6	Ozone + Free Chlorine	50000	5	0	0			39
58	D	21	6	Ozone + Free Chlorine	2000	5	0	0		3.87	39
58	D	21	6	Ozone + Free Chlorine	20000	5	0	0			39
58	D	21	6	Ozone + Free Chlorine	200000	5	1	0.2	3.87		39
58	D	21	6	Chlorine	50	5	0	0		-0.08	39
58	D	21	6	Chlorine	500	5	4	0.8	-0.08		39
58	D	21	6	Chlorine	50	5	1	0.2	0.27	0.34	39

(Continued)

Table B.4 (Continued)

58	D	21	6	Chlorine	500	5	3	0.6	0.40		39
58	D	21	6	Control	50	5	1	0.2	0.27	0.27	39
58	D	21	6	Control	500	5	5	1			39
59	D	21	6	Ozone	100	5	0	0		1.70	39
59	D	21	6	Ozone	1000	5	1	0.2	1.57		39
59	D	21	6	Ozone	10000	5	3	0.6	1.70		39
59	D	21	6	Ozone + Free Chlorine	500	5	0	0		2.10	39
59	D	21	6	Ozone + Free Chlorine	5000	5	2	0.4	1.79		39
59	D	21	6	Ozone + Free Chlorine	50000	5	3	0.6	2.40		39
59	D	21	6	Ozone + Free Chlorine	1000	5	0	0		2.70	39
59	D	21	6	Ozone + Free Chlorine	10000	5	0	0			39
59	D	21	6	Ozone + Free Chlorine	100000	5	3	0.6	2.70		39
59	D	21	6	Chlorine	50	5	1	0.2	0.27	0.10	39
59	D	21	6	Chlorine	500	5	4	0.8	-0.08		39
59	D	21	6	Chlorine	50	5	0	0		0.79	39
59	D	21	6	Chlorine	500	5	2	0.4	0.79		39
59	D	21	6	Control	50	5	0	0		-0.02	39
59	D	21	6	Control	500	5	5	1			39
60	D	21	8	Ozone	100	5	2	0.4	0.09	0.09	39
60	D	21	8	Ozone	1000	5	5	1			39
60	D	21	8	Ozone	10000	5	5	1			39
60	D	21	8	Ozone + Free Chlorine	100	5	0	0		0.28	39
60	D	21	8	Ozone + Free Chlorine	1000	5	5	1			39
60	D	21	8	Ozone + Free Chlorine	10000	5	5	1			39
60	D	21	8	Ozone + Free Chlorine	500	5	3	0.6	0.40	0.40	39
60	D	21	8	Ozone + Free Chlorine	5000	5	5	1			39
60	D	21	8	Ozone + Free Chlorine	50000	5	5	1			39
60	D	21	8	Ozone	100	5	0	0		0.28	39

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
60	D	21	8	Ozone	1000	5	5	1			39
60	D	21	8	Ozone	10000	5	5	1			39
60	D	21	8	Ozone + Free Chlorine	100	5	1	0.2	0.57	0.83	39
60	D	21	8	Ozone + Free Chlorine	1000	5	3	0.6	0.70		39
60	D	21	8	Ozone + Free Chlorine	10000	5	4	0.8	1.23		39
60	D	21	8	Ozone + Free Chlorine	500	5	2	0.4	0.79	1.23	39
60	D	21	8	Ozone + Free Chlorine	5000	5	5	1			39
60	D	21	8	Ozone + Free Chlorine	50000	5	4	0.8	1.92		39
60	D	21	8	Control	50	5	3	0.6	-0.60	-0.73	39
61	D	21	6	Ozone	500	5	0	0		1.40	39
61	D	21	6	Ozone	5000	5	3	0.6	1.40		39
61	D	21	6	Ozone	50000	5	5	1			39
61	D	21	6	Ozone + Free Chlorine	500	5	0	0		2.79	39
61	D	21	6	Ozone + Free Chlorine	5000	5	0	0			39
61	D	21	6	Ozone + Free Chlorine	50000	5	2	0.4	2.79		39
61	D	21	6	Ozone + Free Chlorine	2000	5	1	0.2	1.87	2.53	39
61	D	21	6	Ozone + Free Chlorine	20000	5	0	0			39
61	D	21	6	Ozone + Free Chlorine	200000	5	2	0.4	3.40		39
61	D	21	6	Chlorine	50	5	1	0.2	0.27	0.27	39
61	D	21	6	Chlorine	500	5	5	1			39
61	D	21	6	Chlorine	50	5	1	0.2	0.27	0.53	39
61	D	21	6	Chlorine	500	5	2	0.4	0.79		39
61	D	21	6	Control	50	5	3	0.6	-0.60	-0.73	39
61	D	21	6	Control	500	5	5	1			39
62	D	21	6	Ozone	100	5	0	0		1.14	40
62	D	21	6	Ozone	1000	5	2	0.4	1.14		40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
62	D	21	6	Ozone	10000	5	5	1			40
62	D	21	6	Ozone + Free Chlorine	100	5	0	0		1.14	40
62	D	21	6	Ozone + Free Chlorine	1000	5	2	0.4	1.14		40
62	D	21	6	Ozone + Free Chlorine	10000	5	5	1			40
62	D	21	6	Ozone + Free Chlorine	500	5	1	0.2	1.21	1.37	40
62	D	21	6	Ozone + Free Chlorine	5000	5	3	0.6	1.54		40
62	D	21	6	Ozone + Free Chlorine	50000	5	5	1			40
62	D	21	6	Chlorine	50	5	1	0.2	0.21	0.37	40
62	D	21	6	Chlorine	500	5	3	0.6	0.54		40
62	D	21	6	Chlorine	50	5	2	0.4	-0.16	0.19	40
62	D	21	6	Chlorine	500	5	3	0.6	0.54		40
62	D	21	6	Control	50	5	3	0.6	-0.46	-0.46	40
62	D	21	6	Control	500	5	5	1			40
63	D	2	8	Ozone	500	5	3	0.6	0.54	0.54	40
63	D	2	8	Ozone	5000	5	5	1			40
63	D	2	8	Ozone	50000	5	5	1			40
63	D	2	8	Ozone + Free Chlorine	500	5	5	1		<0.17	40
63	D	2	8	Ozone + Free Chlorine	5000	5	5	1			40
63	D	2	8	Ozone + Free Chlorine	50000	5	5	1			40
63	D	2	8	Ozone + Free Chlorine	1000	5	3	0.6	0.84	0.84	40
63	D	2	8	Ozone + Free Chlorine	10000	5	5	1			40
63	D	2	8	Ozone + Free Chlorine	100000	5	5	1			40
63	D	2	8	Chlorine	50	5	2	0.4	-0.16	-0.16	40
63	D	2	8	Chlorine	500	5	5	1			40
63	D	2	8	Chlorine	50	5	2	0.4	-0.16	0.19	40
63	D	2	8	Chlorine	500	5	3	0.6	0.54		40
63	D	2	8	Control	50	5	2	0.4	-0.16	-0.16	40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
63	D	2	8	Control	500	5	5	1			40
64	D	4.8	8	Ozone	100	5	4	0.8	-0.52	-0.52	40
64	D	4.8	8	Ozone	1000	5	5	1			40
64	D	4.8	8	Ozone	10000	5	5	1			40
64	D	4.8	8	Ozone + Free Chlorine	100	5	3	0.6	-0.16	-0.16	40
64	D	4.8	8	Ozone + Free Chlorine	1000	5	5	1			40
64	D	4.8	8	Ozone + Free Chlorine	10000	5	5	1			40
64	D	4.8	8	Ozone + Free Chlorine	200	5	3	0.6	0.14	0.14	40
64	D	4.8	8	Ozone + Free Chlorine	2000	5	5	1			40
64	D	4.8	8	Ozone + Free Chlorine	20000	5	5	1			40
64	D	4.8	8	Chlorine	50	5	3	0.6	-0.46	-0.46	40
64	D	4.8	8	Chlorine	500	5	5	1			40
64	D	4.8	8	Chlorine	50	5	3	0.6	-0.46	-0.46	40
64	D	4.8	8	Chlorine	500	5	5	1			40
64	D	4.8	8	Control	50	5	3	0.6	-0.46	-0.46	40
64	D	4.8	8	Control	500	5	5	1			40
65	D	4.9	8	Ozone	100	5	4	0.8	-0.52	-0.52	40
65	D	4.9	8	Ozone	1000	5	5	1			40
65	D	4.9	8	Ozone	10000	5	5	1			40
65	D	4.9	8	Ozone + Free Chlorine	100	5	5	1		0.48	40
65	D	4.9	8	Ozone + Free Chlorine	1000	5	4	0.8	0.48		40
65	D	4.9	8	Ozone + Free Chlorine	10000	5	5	1			40
65	D	4.9	8	Ozone + Free Chlorine	200	5	4	0.8	-0.22	-0.22	40
65	D	4.9	8	Ozone + Free Chlorine	2000	5	5	1			40
65	D	4.9	8	Ozone + Free Chlorine	20000	5	5	1			40
65	D	4.9	8	Chlorine	50	5	3	0.6	-0.46	-0.46	40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
65	D	4.9	8	Chlorine	500	5	5	1			40
65	D	4.9	8	Chlorine	50	5	3	0.6	-0.46	0.04	40
65	D	4.9	8	Chlorine	500	5	3	0.6	0.54		40
65	D	4.9	8	Control	50	5	1	0.2	0.21	0.21	40
65	D	4.9	8	Control	500	5	5	1			40
66	D	21	8	Ozone	100	5	2	0.4	0.14	0.64	40
66	D	21	8	Ozone	1000	5	2	0.4	1.14		40
66	D	21	8	Ozone	10000	5	5	1			40
66	D	21	8	Ozone + Monochloramine	100	5	0	0		2.14	40
66	D	21	8	Ozone + Monochloramine	1000	5	0	0			40
66	D	21	8	Ozone + Monochloramine	10000	5	2	0.4	2.14		40
66	D	21	8	Ozone + Monochloramine	100	5	0	0		>2.51	40
66	D	21	8	Ozone + Monochloramine	1000	5	0	0			40
66	D	21	8	Ozone + Monochloramine	10000	5	1	0.2	2.51		40
66	D	21	8	Monochloramine	50	5	1	0.2	0.21	0.19	40
66	D	21	8	Monochloramine	500	5	4	0.8	0.17		40
66	D	21	8	Monochloramine	50	5	0	0		>1.21	40
66	D	21	8	Monochloramine	500	5	0	0			40
66	D	21	8	Control	50	5	3	0.6	-0.46	-0.46	40
67	D	21	8	Ozone	100	5	1	0.2	0.51	1.01	40
67	D	21	8	Ozone	1000	5	1	0.2	1.51		40
67	D	21	8	Ozone	10000	5	5	1			40
67	D	21	8	Ozone + Monochloramine	200	5	0	0		1.80	40
67	D	21	8	Ozone + Monochloramine	2000	5	0	0			40
67	D	21	8	Ozone + Monochloramine	20000	5	5	1			40
67	D	21	8	Ozone + Monochloramine	1000	5	1	0.2	1.51	1.68	40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
67	D	21	8	Ozone + Monochloramine	10000	5	3	0.6	1.84		40
67	D	21	8	Ozone + Monochloramine	100000	5	5	1			40
67	D	21	8	Monochloramine	100	5	1	0.2	0.51	0.68	40
67	D	21	8	Monochloramine	1000	5	3	0.6	0.84		40
67	D	21	8	Monochloramine	100	5	1	0.2	0.51	0.68	40
67	D	21	8	Monochloramine	1000	5	3	0.6	0.84		40
67	D	21	8	Control	50	5	2	0.4	-0.16	-0.16	40
67	D	21	8	Control	500	5	5	1			40
68	D	21	8	Ozone	100	5	4	0.8	-0.52	-0.52	40
68	D	21	8	Ozone	1000	5	5	1			40
68	D	21	8	Ozone	10000	5	5	1			40
68	D	21	8	Ozone + Monochloramine	200	5	0	0		1.61	40
68	D	21	8	Ozone + Monochloramine	2000	5	2	0.4	1.44		40
68	D	21	8	Ozone + Monochloramine	20000	5	4	0.8	1.78		40
68	D	21	8	Ozone + Monochloramine	1000	5	0	0		2.49	40
68	D	21	8	Ozone + Monochloramine	10000	5	1	0.2	2.51		40
68	D	21	8	Ozone + Monochloramine	100000	5	4	0.8	2.48		40
68	D	21	8	Monochloramine	100	5	1	0.2	0.51	0.51	40
68	D	21	8	Monochloramine	1000	5	5	1			40
68	D	21	8	Monochloramine	100	5	1	0.2	0.51	0.68	40
68	D	21	8	Monochloramine	1000	5	3	0.6	0.84		40
68	D	21	8	Control	50	5	1	0.2	0.21	0.19	40
68	D	21	8	Control	500	5	4	0.8	0.17		40
69	D	6	6	Ozone	100	5	3	0.6	-0.16	-0.16	40
69	D	6	6	Ozone	1000	5	5	1			40
69	D	6	6	Ozone	10000	5	5	1			40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
69	D	6	6	Ozone + Free Chlorine	200	5	0	0		0.78	40
69	D	6	6	Ozone + Free Chlorine	2000	5	4	0.8	0.78		40
69	D	6	6	Ozone + Free Chlorine	20000	5	5	1			40
69	D	6	6	Ozone + Free Chlorine	1000	5	2	0.4	1.14	1.14	40
69	D	6	6	Ozone + Free Chlorine	10000	5	5	1			40
69	D	6	6	Ozone + Free Chlorine	100000	5	5	1			40
69	D	6	6	Chlorine	50	5	1	0.2	0.21	0.21	40
69	D	6	6	Chlorine	500	5	5	1			40
69	D	6	6	Chlorine	50	5	2	0.4	-0.16	0.01	40
69	D	6	6	Chlorine	500	5	4	0.8	0.17		40
69	D	6	6	Control	50	5	4	0.8	-0.83	-0.83	40
69	D	6	6	Control	500	4	4	1			40
70	D	6	6	Ozone	100	5	1	0.2	0.51	0.49	40
70	D	6	6	Ozone	1000	5	4	0.8	0.48		40
70	D	6	6	Ozone	10000	5	5	1			40
70	D	6	6	Ozone + Free Chlorine	200	5	1	0.2	0.81	0.79	40
70	D	6	6	Ozone + Free Chlorine	2000	5	4	0.8	0.78		40
70	D	6	6	Ozone + Free Chlorine	20000	5	5	1			40
70	D	6	6	Ozone + Free Chlorine	1000	5	2	0.4	1.14	1.31	40
70	D	6	6	Ozone + Free Chlorine	10000	5	4	0.8	1.48		40
70	D	6	6	Ozone + Free Chlorine	100000	5	5	1			40
70	D	6	6	Chlorine	50	5	1	0.2	0.21	0.21	40
70	D	6	6	Chlorine	50	5	2	0.4	-0.16	0.01	40
70	D	6	6	Chlorine	500	5	4	0.8	0.17		40
71	D	21	6	Ozone	100	5	0	0		2.51	40
71	D	21	6	Ozone	1000	5	0	0			40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
71	D	21	6	Ozone	10000	5	1	0.2	2.51		40
71	D	21	6	Ozone + Monochloramine	500	5	0	0		2.54	40
71	D	21	6	Ozone + Monochloramine	5000	5	0	0			40
71	D	21	6	Ozone + Monochloramine	50000	5	3	0.6	2.54		40
71	D	21	6	Ozone + Monochloramine	1000	5	0	0		3.14	40
71	D	21	6	Ozone + Monochloramine	10000	5	0	0			40
71	D	21	6	Ozone + Monochloramine	100000	5	2	0.4	3.14		40
71	D	21	6	Monochloramine	50	5	0	0		0.17	40
71	D	21	6	Monochloramine	500	5	4	0.8	0.17		40
71	D	21	6	Monochloramine	50	5	1	0.2	0.21	0.37	40
71	D	21	6	Monochloramine	500	5	3	0.6	0.54		40
71	D	21	6	Control	50	5	2	0.4	-0.16	-0.16	40
	D										
72	D	5	6	Ozone	100	5	5	1		<-.52	40
72	D	5	6	Ozone	1000	5	5	1			40
72	D	5	6	Ozone	10000	5	5	1			40
72	D	5	6	Ozone + Monochloramine	200	5	5	1		<-0.22	40
72	D	5	6	Ozone + Monochloramine	2000	5	5	1			40
72	D	5	6	Ozone + Monochloramine	20000	5	5	1			40
72	D	5	6	Ozone + Monochloramine	500	5	3	0.6	0.54	0.67	40
72	D	5	6	Ozone + Monochloramine	5000	5	5	1			40
72	D	5	6	Ozone + Monochloramine	50000	5	5	1			40
72	D	5	6	Monochloramine	50	5	1	0.2	0.21	0.21	40
72	D	5	6	Monochloramine	500	5	5	1			40
72	D	5	6	Monochloramine	50	5	1	0.2	0.21	0.21	40
72	D	5	6	Monochloramine	500	5	5	1			40
72	D	5	6	Control	50	5	2	0.4	-0.16	-0.06	40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
73	D	5	8	Ozone	100	5	0	0		1.14	40
73	D	5	8	Ozone	1000	5	2	0.4	1.14		40
73	D	5	8	Ozone	10000	5	5	1			40
73	D	5	8	Ozone + Monochloramine	500	5	2	0.4	0.84	1.19	40
73	D	5	8	Ozone + Monochloramine	5000	5	3	0.6	1.54		40
73	D	5	8	Ozone + Monochloramine	50000	5	5	1			40
73	D	5	8	Ozone + Monochloramine	1000	5	0	0		2.31	40
73	D	5	8	Ozone + Monochloramine	10000	5	2	0.4	2.14		40
73	D	5	8	Ozone + Monochloramine	100000	5	4	0.8	2.48		40
73	D	5	8	Monochloramine	50	5	2	0.4	-0.16	0.01	40
73	D	5	8	Monochloramine	500	5	4	0.8	0.17		40
73	D	5	8	Monochloramine	50	5	2	0.4	-0.16	0.01	40
73	D	5	8	Monochloramine	500	5	4	0.8	0.17		40
73	D	5	8	Control	50	5	1	0.2	0.21	0.28	40
74	D	21	6	Ozone	500	5	1	0.2	1.21	1.21	40
74	D	21	6	Ozone	5000	5	5	1			40
74	D	21	6	Ozone	50000	5	5	1			40
74	D	21	6	Ozone + Monochloramine	500	5	0	0		>3.21	40
74	D	21	6	Ozone + Monochloramine	5000	5	0	0			40
74	D	21	6	Ozone + Monochloramine	50000	5	0	0			40
74	D	21	6	Ozone + Monochloramine	1000	5	0	0		>3.51	40
74	D	21	6	Ozone + Monochloramine	10000	5	0	0			40
74	D	21	6	Ozone + Monochloramine	100000	5	0	0			40
74	D	21	6	Monochloramine	50	5	1	0.2	0.21	0.21	40
74	D	21	6	Monochloramine	500	5	5	1			40
74	D	21	6	Monochloramine	50	5	0	0		0.19	40
74	D	21	6	Monochloramine	500	5	5	1			40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
74	D	21	6	Control	50	5	1	0.2	0.21	0.21	40
75	D	21	6	Ozone	500	5	0	0		2.21	40
75	D	21	6	Ozone	5000	5	1	0.2	2.21		40
75	D	21	6	Ozone	50000	5	5	1			40
75	D	21	6	Ozone + Monochloramine	500	5	0	0		>3.21	40
75	D	21	6	Ozone + Monochloramine	5000	5	0	0			40
75	D	21	6	Ozone + Monochloramine	50000	5	0	0			40
75	D	21	6	Ozone + Monochloramine	1000	5	0	0		>3.51	40
75	D	21	6	Ozone + Monochloramine	10000	5	0	0			40
75	D	21	6	Ozone + Monochloramine	100000	5	0	0			40
75	D	21	6	Monochloramine	50	5	0	0		0.19	40
75	D	21	6	Monochloramine	500	5	5	1			40
75	D	21	6	Monochloramine	50	5	0	0		1.21	40
75	D	21	6	Monochloramine	500	5	1	0.2	1.21		40
75	D	21	6	Control	50	5	1	0.2	0.21	0.21	40
76	D	7	6	Ozone	100	5	0	0		0.84	40
76	D	7	6	Ozone	1000	5	3	0.6	0.84		40
76	D	7	6	Ozone	10000	5	5	1			40
76	D	7	6	Ozone + Monochloramine	500	5	0	0		2.54	40
76	D	7	6	Ozone + Monochloramine	5000	5	0	0			40
76	D	7	6	Ozone + Monochloramine	50000	5	3	0.6	2.54		40
76	D	7	6	Ozone + Monochloramine	1000	5	0	0		2.84	40
76	D	7	6	Ozone + Monochloramine	10000	5	0	0			40
76	D	7	6	Ozone + Monochloramine	100000	5	3	0.6	2.84		40
76	D	7	6	Monochloramine	50	5	1	0.2	0.21	0.37	40
76	D	7	6	Monochloramine	500	5	3	0.6	0.54		40

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
76	D	7	6	Monochloramine	50	5	0	0		0.54	40
76	D	7	6	Monochloramine	500	5	3	0.6	0.54		40
76	D	7	6	Control	50	5	5	1		Suspected	40
76	D	7	6	Control	500	5	1	0.2	1.21	mixup	40
77	D	7	8	Ozone	100	5	1	0.2	0.51	1.16	40
77	D	7	8	Ozone	1000	5	2	0.4	1.14		40
77	D	7	8	Ozone	10000	5	3	0.6	1.84		40
77	D	7	8	Ozone + Monochloramine	500	5	0	0		2.84	40
77	D	7	8	Ozone + Monochloramine	5000	5	0	0			40
77	D	7	8	Ozone + Monochloramine	50000	5	2	0.4	2.84		40
77	D	7	8	Ozone + Monochloramine	1000	5	0	0		3.51	40
77	D	7	8	Ozone + Monochloramine	10000	5	0	0			40
77	D	7	8	Ozone + Monochloramine	100000	5	1	0.2	3.51		40
77	D	7	8	Monochloramine	50	5	1	0.2	0.21	0.37	40
77	D	7	8	Monochloramine	500	5	3	0.6	0.54		40
77	D	7	8	Monochloramine	50	5	0	0		0.84	40
77	D	7	8	Monochloramine	500	5	2	0.4	0.84		40
77	D	7	8	Control	50	5	1	0.2	0.21	0.53	40
77	D	7	8	Control	500	5	2	0.4	0.84		40
78	D	1	8	Ozone	100	5	3	0.6	-0.16	-0.16	41
78	D	1	8	Ozone	1000	5	5	1			41
78	D	1	8	Ozone	10000	5	5	1			41
78	D	1	8	Ozone + Monochloramine	500	5	1	0.2	1.09	1.09	41
78	D	1	8	Ozone + Monochloramine	5000	5	5	1			41
78	D	1	8	Ozone + Monochloramine	50000	5	5	1			41
78	D	1	8	Ozone + Monochloramine	1000	5	4	0.8	0.54	0.54	41

(Continued)

Table B.4 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
78	D	1	8	Ozone + Monochloramine	10000	5	5	1			41
78	D	1	8	Ozone + Monochloramine	100000	5	5	1			41
78	D	1	8	Monochloramine	50	5	1	0.2	0.09	0.09	41
78	D	1	8	Monochloramine	500	5	5	1			41
78	D	1	8	Monochloramine	50	5	5	1		<-0.76	41
78	D	1	8	Monochloramine	500	5	5	1			41
78	D	1	8	Control	50	5	3	0.6	-0.46	-0.46	41
78	D	1	8	Control	500	5	5	1			41
79	D	1	6	Ozone	100	5	5	1		<-0.46	41
79	D	1	6	Ozone	1000	4	4	1			41
79	D	1	6	Ozone	10000	5	5	1			41
79	D	1	6	Ozone + Monochloramine	300	5	5	1		1.02	41
79	D	1	6	Ozone + Monochloramine	3000	5	4	0.8	1.02		41
79	D	1	6	Ozone + Monochloramine	30000	5	5	1			41
79	D	1	6	Ozone + Monochloramine	500	5	4	0.8	0.24	0.24	41
79	D	1	6	Ozone + Monochloramine	5000	5	5	1			41
79	D	1	6	Ozone + Monochloramine	50000	5	5	1			41
79a	D	1	6	Ozone	100	5	5	1		<-0.46	41
79a	D	1	6	Ozone	1000	5	5	1			41
79a	D	1	6	Ozone	10000	5	5	1			41
79a	D	1	6	Ozone + Monochloramine	500	5	4	0.8	0.24	0.24	41
79a	D	1	6	Ozone + Monochloramine	5000	5	5	1			41
79a	D	1	6	Ozone + Monochloramine	50000	5	5	1			41
79a	D	1	6	Ozone + Monochloramine	1000	5	5	1		<0.54	41
79a	D	1	6	Ozone + Monochloramine	10000	5	5	1			41
79a	D	1	6	Ozone + Monochloramine	100000	5	5	1			41

Table B.5

Neonatal CD-1 infectivity analysis for experimental trials with natural water E

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
80	E	21	8	Ozone	200	5	1	0.2	0.69	1.41	41
80	E	21	8	Ozone	2000	5	0	0			41
80	E	21	8	Ozone	20000	5	3	0.6	2.14		41
80	E	21	8	Ozone + Free Chlorine	500	5	1	0.2	1.09	1.80	41
80	E	21	8	Ozone + Free Chlorine	5000	5	1	0.2	2.09		41
80	E	21	8	Ozone + Free Chlorine	50000	5	4	0.8	2.24		41
80	E	21	8	Ozone + Free Chlorine	1000	5	0	0		1.84	41
80	E	21	8	Ozone + Free Chlorine	10000	5	3	0.6	1.84		41
80	E	21	8	Ozone + Free Chlorine	100000	5	5	1			41
80	E	21	8	Chlorine	50	5	4	0.8	-0.76	-0.76	41
80	E	21	8	Chlorine	500	5	5	1			41
80	E	21	8	Chlorine	50	5	3	0.6	-0.46	-0.46	41
80	E	21	8	Chlorine	500	5	5	1			41
80	E	21	8	Control	50	5	5	1			41
80	E	21	8	Control	500	5	0	0			41
81	D	1	6	Ozone	50	5	2	0.4	-0.21	0.01	41
81	D	1	6	Ozone	500	5	4	0.8	0.24		41
81	D	1	6	Ozone	5000	5	5	1			41
81	D	1	6	Ozone + Chlorine	50	5	0	0		0.17	41
81	D	1	6	Ozone + Chlorine	500	5	5	1			41
81	D	1	6	Ozone + Chlorine	5000	5	5	1			41
81	D	1	6	Ozone + Chlorine	100	5	2	0.4	0.09	0.59	41
81	D	1	6	Ozone + Chlorine	1000	5	2	0.4	1.09		41
81	D	1	6	Ozone + Chlorine	10000	5	5	1			41
81	D	1	6	Chlorine	50	5	2	0.4	-0.21	-0.21	41
81	D	1	6	Chlorine	500	5	5	1			41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
81	D	1	6	Chlorine	50	5	4	0.8	-0.76	-0.76	41
81	D	1	6	Chlorine	500	5	5	1			41
81	D	1	6	Control	50	5	4	0.8	-0.76	-0.76	41
81	D	1	6	Control	500	5	5	1			41
82	E	1	8	Ozone	100	5	2	0.4	0.09	0.31	41
82	E	1	8	Ozone	1000	5	4	0.8	0.54		41
82	E	1	8	Ozone	10000	5	5	1			41
82	E	1	8	Ozone + Chlorine	500	5	4	0.8	0.24	0.24	41
82	E	1	8	Ozone + Chlorine	5000	5	5	1			41
82	E	1	8	Ozone + Chlorine	50000	5	5	1			41
82	E	1	8	Ozone + Chlorine	500	5	2	0.4	0.79	1.01	41
82	E	1	8	Ozone + Chlorine	5000	5	4	0.8	1.24		41
82	E	1	8	Ozone + Chlorine	50000	5	5	1			41
82	E	1	8	Chlorine	50	5	1	0.2	0.09	0.09	41
82	E	1	8	Chlorine	500	5	5	1			41
82	E	1	8	Chlorine	50	5	3	0.6	-0.46	-0.46	41
82	E	1	8	Chlorine	500	5	5	1			41
82	E	1	8	Control	50	5	4	0.8	-0.76	-0.76	41
82	E	1	8	Control	500	5	5	1			41
83	E	1	8	Ozone	50	5	1	0.2	0.09	0.09	41
83	E	1	8	Ozone	500	5	5	1			41
83	E	1	8	Ozone	5000	5	5	1			41
83	E	1	8	Ozone + Monochloramine	100	5	2	0.4	0.09	0.09	41
83	E	1	8	Ozone + Monochloramine	1000	5	5	1			41
83	E	1	8	Ozone + Monochloramine	10000	5	5	1			41
83	E	1	8	Ozone + Monochloramine	500	5	3	0.6	0.54	0.54	41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
83	E	1	8	Ozone + Monochloramine	5000	5	5	1			41
83	E	1	8	Ozone + Monochloramine	50000	5	5	1			41
83	E	1	8	Monochloramine	50	5	5	1		<-0.76	41
83	E	1	8	Monochloramine	500	5	5	1			41
83	E	1	8	Monochloramine	50	5	4	0.8	-0.76	-0.76	41
83	E	1	8	Monochloramine	500	5	5	1			41
83	E	1	8	Control	50	5	3	0.6	-0.46	-0.46	41
83	E	1	8	Control	500	5	5	1			41
84	E	21	6	Ozone	80	5	0	0		0.99	41
84	E	21	6	Ozone	800	5	2	0.4	0.99		41
84	E	21	6	Ozone	8000	5	5	1			41
84	E	21	6	Ozone + Monochloramine	100	5	0	0		>2.39	41
84	E	21	6	Ozone + Monochloramine	1000	5	0	0			41
84	E	21	6	Ozone + Monochloramine	10000	5	0	0			41
84	E	21	6	Ozone + Monochloramine	500	5	0	0		>3	41
84	E	21	6	Ozone + Monochloramine	5000	5	0	0			41
84	E	21	6	Ozone + Monochloramine	50000	4	0	0			41
84	E	21	6	Monochloramine	50	5	4	0.8	-0.76	-0.76	41
84	E	21	6	Monochloramine	500	5	5	1			41
84	E	21	6	Monochloramine	50	5	1	0.2	0.09	0.09	41
84	E	21	6	Monochloramine	500	5	5	1			41
84	E	21	6	Control	50	5	4	0.8	-0.76	-0.76	41
84	E	21	6	Control	500	5	5	1			41
85	E	21	6	Ozone	500	5	0	0		3.09	41
85	E	21	6	Ozone	5000	5	0	0			41
85	E	21	6	Ozone	50000	5	1	0.2	3.09		41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
85	E	21	6	Ozone + Chlorine	1000	5	0	0		2.89	41
85	E	21	6	Ozone + Chlorine	10000	5	1	0.2	2.39		41
85	E	21	6	Ozone + Chlorine	100000	5	1	0.2	3.39		41
85	E	21	6	Ozone + Chlorine	1000	5	0	0		>3.39	41
85	E	21	6	Ozone + Chlorine	10000	5	0	0			41
85	E	21	6	Ozone + Chlorine	100000	5	0	0			41
85	E	21	6	Chlorine	50	5	2	0.4	-0.21	-0.21	41
85	E	21	6	Chlorine	500	5	5	1			41
85	E	21	6	Chlorine	50	5	0	0		1.09	41
85	E	21	6	Chlorine	500	5	1	0.2	1.09		41
85	E	21	6	Control	50	5	3	0.6	-0.46	-0.46	41
85	E	21	6	Control	500	5	5	1			41
86	E	21	8	Ozone	300	5	0	0		1.79	41
86	E	21	8	Ozone	3000	5	2	0.4	1.56		41
86	E	21	8	Ozone	30000	5	4	0.8	2.02		41
86	E	21	8	Ozone + Monochloramine	1000	5	0	0		>3.39	41
86	E	21	8	Ozone + Monochloramine	10000	5	0	0			41
86	E	21	8	Ozone + Monochloramine	100000	5	0	0			41
86	E	21	8	Ozone + Monochloramine	1000	5	0	0		>3.39	41
86	E	21	8	Ozone + Monochloramine	10000	5	0	0			41
86	E	21	8	Ozone + Monochloramine	100000	5	0	0			41
86	E	21	8	Monochloramine	50	5	0	0		0.17	41
86	E	21	8	Monochloramine	500	5	5	1			41
86	E	21	8	Monochloramine	50	5	0	0		0.79	41
86	E	21	8	Monochloramine	500	5	2	0.4	0.79		41
86	E	21	8	Control	50	5	0	0		0.17	41
86	E	21	8	Control	500	5	5	1			41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
87	E	1	6	Ozone	500	5	2	0.4	0.79	1.29	41
87	E	1	6	Ozone	5000	5	2	0.4	1.79		41
87	E	1	6	Ozone	50000	5	5	1			41
87	E	1	6	Ozone + Monochloramine	1000	5	1	0.2	1.39	1.39	41
87	E	1	6	Ozone + Monochloramine	10000	5	0	0			41
87	E	1	6	Ozone + Monochloramine	100000	5	5	1			41
87	E	1	6	Ozone + Monochloramine	1000	5	0	0		2.89	41
87	E	1	6	Ozone + Monochloramine	10000	5	1	0.2	2.39		41
87	E	1	6	Ozone + Monochloramine	100000	5	1	0.2	3.39		41
87	E	1	6	Control	50	5	2	0.4	-0.21	-0.21	41
87	E	1	6	Control	500	na	5				41
88	E	1	6	Ozone	50	5	3	0.6	-0.46	-0.11	41
88	E	1	6	Ozone	500	5	4	0.8	0.24		41
88	E	1	6	Ozone	5000	5	5	1			41
88	E	1	6	Ozone + Chlorine	100	5	2	0.4	0.09	0.09	41
88	E	1	6	Ozone + Chlorine	1000	5	5	1			41
88	E	1	6	Ozone + Chlorine	10000	5	5	1			41
88	E	1	6	Ozone + Chlorine	100	5	1	0.2	0.39	0.46	41
88	E	1	6	Ozone + Chlorine	1000	5	4	0.8	0.54		41
88	E	1	6	Ozone + Chlorine	10000	5	5	1			41
88	E	1	6	Chlorine	50	5	2	0.4	-0.21	0.01	41
88	E	1	6	Chlorine	500	5	4	0.8	0.24		41
88	E	1	6	Chlorine	50	5	3	0.6	-0.46	-0.11	41
88	E	1	6	Chlorine	500	5	4	0.8	0.24		41
88	E	1	6	Control	50	5	na			0.54	41
88	E	1	6	Control	500	5	3	0.6	0.54		41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
89	E	1	8	Ozone	50	5	2	0.4	-0.21	-0.21	41
89	E	1	8	Ozone	500	5	5	1			41
89	E	1	8	Ozone	5000	5	5	1			41
89	E	1	8	Ozone + Monochloramine	100	5	0	0		0.84	41
89	E	1	8	Ozone + Monochloramine	1000	5	3	0.6	0.84		41
89	E	1	8	Ozone + Monochloramine	10000	5	5	1			41
89	E	1	8	Ozone + Monochloramine	100	5	2	0.4	0.09	0.31	41
89	E	1	8	Ozone + Monochloramine	1000	5	4	0.8	0.54		41
89	E	1	8	Ozone + Monochloramine	10000	5	5	1			41
89	E	1	8	Monochloramine	50	5	4	0.8	-0.76	-0.26	41
89	E	1	8	Monochloramine	500	5	4	0.8	0.24		41
89	E	1	8	Monochloramine	50	5	4	0.8	-0.76	-0.76	41
89	E	1	8	Monochloramine	500	5	5	1			41
89	E	1	8	Control	50	5	1	0.2	0.09	0.16	41
89	E	1	8	Control	500	5	4	0.8	0.24		41
90	E	1	6	Ozone	500	5	0	0		1.54	41
90	E	1	6	Ozone	5000	5	3	0.6	1.54		41
90	E	1	6	Ozone	50000	5	5	1			41
90	E	1	6	Ozone + Monochloramine	1000	5	0	0		2.54	41
90	E	1	6	Ozone + Monochloramine	10000	5	0	0			41
90	E	1	6	Ozone + Monochloramine	100000	5	4	0.8	2.54		41
90	E	1	6	Ozone + Monochloramine	2000	5	0	0		>3.69	41
90	E	1	6	Ozone + Monochloramine	20000	5	0	0			41
90	E	1	6	Ozone + Monochloramine	200000	5	0	0			41
90	E	1	6	Monochloramine	50	5	1	0.2	0.09	0.09	41
90	E	1	6	Monochloramine	500	5	5	1			41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
90	E	1	6	Monochloramine	50	5	3	0.6	-0.46	-0.46	41
90	E	1	6	Monochloramine	500	5	5	1			41
90	E	1	6	Control	50	5	0	0		0.79	41
90	E	1	6	Control	500	5	2	0.4	0.79		41
91	E	1	8	Ozone	200	5	0	0		1.69	41
91	E	1	8	Ozone	2000	5	1	0.2	1.69		41
91	E	1	8	Ozone	20000	5	5	1			41
91	E	1	8	Ozone + Chlorine	500	5	2	0.4	0.79	1.16	41
91	E	1	8	Ozone + Chlorine	5000	5	3	0.6	1.54		41
91	E	1	8	Ozone + Chlorine	50000	5	5	1			41
91	E	1	8	Ozone + Chlorine	500	5	0	0		1.89	41
91	E	1	8	Ozone + Chlorine	5000	5	3	0.6	1.54		41
91	E	1	8	Ozone + Chlorine	50000	5	4	0.8	2.24		41
91	E	1	8	Chlorine	50	5	3	0.6	-0.46	-0.46	41
91	E	1	8	Chlorine	50	5	0	0		0.24	41
91	E	1	8	Chlorine	500	5	4	0.8	0.24		41
92	E	1	8	Ozone	50	5	0	0		0.17	41
92	E	1	8	Ozone	500	5	5	1			41
92	E	1	8	Ozone	5000	5	5	1			41
92	E	1	8	Ozone + Monochloramine	100	5	1	0.2	0.39	0.61	41
92	E	1	8	Ozone + Monochloramine	1000	5	3	0.6	0.84		41
92	E	1	8	Ozone + Monochloramine	10000	5	5	1			41
92	E	1	8	Ozone + Monochloramine	200	5	1	0.2	0.69	0.76	41
92	E	1	8	Ozone + Monochloramine	2000	5	4	0.8	0.84		41
92	E	1	8	Ozone + Monochloramine	20000	5	5	1			41
92	E	1	8	Monochloramine	50	5	3	0.6	-0.46	-0.46	41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
92	E	1	8	Monochloramine	500	5	5	1			41
92	E	1	8	Monochloramine	50	5	2	0.4	-0.21	-0.21	41
92	E	1	8	Monochloramine	500	5	5	1			41
92	E	1	8	Control	50	5	2	0.4	-0.21	0.16	41
92	E	1	8	Control	500	5	3	0.6	0.54		41
93	E	21	6	Ozone	100	5	1	0.2	0.39	1.24	41
93	E	21	6	Ozone	1000	5	0	0			41
93	E	21	6	Ozone	10000	5	2	0.4	2.09		41
93	E	21	6	Ozone + Monochloramine	10000	5	0	0		4.39	41
93	E	21	6	Ozone + Monochloramine	100000	5	0	0			41
93	E	21	6	Ozone + Monochloramine	1000000	5	1	0.2	4.39		41
94	E	21	8	Ozone	80	5	0	0		1.74	41
94	E	21	8	Ozone	800	5	0	0			41
94	E	21	8	Ozone	8000	5	3	0.6	1.74		41
94	E	21	8	Ozone + Chlorine	100	5	0	0		2.39	41
94	E	21	8	Ozone + Chlorine	1000	5	0	0			41
94	E	21	8	Ozone + Chlorine	10000	5	1	0.2	2.39		41
94	E	21	8	Ozone + Chlorine	150	5	0	0		2.56	41
94	E	21	8	Ozone + Chlorine	1500	5	0	0			41
94	E	21	8	Ozone + Chlorine	15000	5	1	0.2	2.56		41
94	E	21	8	Chlorine	50	5	0	0		0.54	41
94	E	21	8	Chlorine	500	5	3	0.6	0.54		41
94	E	21	8	Chlorine	50	5	3	0.6	-0.46	-0.11	41
94	E	21	8	Chlorine	500	5	4	0.8	0.24		41
94	E	21	8	Control	100	5	3	0.6	-0.16	-0.16	41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
95	E	21	6	Ozone	1000	5	1	0.2	1.39	2.29	41
95	E	21	6	Ozone	10000	5	2	0.4	2.09		41
95	E	21	6	Ozone	100000	5	1	0.2	3.39		41
95	E	21	6	Ozone + Chlorine	2000	5	0	0		3.19	41
95	E	21	6	Ozone + Chlorine	20000	5	1	0.2	2.69		41
95	E	21	6	Ozone + Chlorine	200000	5	1	0.2	3.69		41
95	E	21	6	Ozone + Chlorine	5000	5	0	0		>4.09	41
95	E	21	6	Ozone + Chlorine	50000	5	0	0			41
95	E	21	6	Ozone + Chlorine	500000	5	0	0			41
95	E	21	6	Chlorine	150	5	3	0.6	0.01	0.01	41
95	E	21	6	Chlorine	50	5	0	0		1.09	41
95	E	21	6	Chlorine	500	5	1	0.2	1.09		41
95	E	21	6	Control	100	5	4	0.8	-0.46	-0.46	41
96	E	21	6	Ozone	100	5	0	0		1.84	41
96	E	21	6	Ozone	1000	4	1	0.25	1.30		41
96	E	21	6	Ozone	10000	5	1	0.2	2.39		41
96	E	21	6	Ozone + Monochloramine	10 000	5	0	0		4.09	41
96	E	21	6	Ozone + Monochloramine	100 000	5	0	0			41
96	E	21	6	Ozone + Monochloramine	1000000	5	2	0.4	4.09		41
96	E	21	6	Monochloramine	150	5	2	0.4	0.26	0.26	41
96	E	21	6	Control	100	5	3	0.6	-0.16	-0.16	41
97	E	21	6	Ozone	1000	5	0	0		2.46	41
97	E	21	6	Ozone	10000	5	2	0.4	2.09		41
97	E	21	6	Ozone	100000	5	3	0.6	2.84		41
97	E	21	6	Ozone + Chlorine	2000	5	0	0		>3.69	41
97	E	21	6	Ozone + Chlorine	20000	5	0	0			41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
97	E	21	6	Ozone + Chlorine	200000	5	0	0			41
97	E	21	6	Ozone + Chlorine	5000	5	0	0		4.09	41
97	E	21	6	Ozone + Chlorine	50000	5	0	0			41
97	E	21	6	Ozone + Chlorine	500000	5	1	0.2	4.09		41
97	E	21	6	Chlorine	150	5	3	0.6	0.01	0.01	41
97	E	21	6	Control	100	5	4	0.8	-0.46	-0.46	41
98	E	21	6	Ozone	50	5	0	0		0.54	41
98	E	21	6	Ozone	500	5	3	0.6	0.54		41
98	E	21	6	Ozone	5 000	5	5	1			41
98	E	21	6	Ozone + Chlorine	100	5	0	0		1.19	41
98	E	21	6	Ozone + Chlorine	1000	5	3	0.6	0.84		41
98	E	21	6	Ozone + Chlorine	10000	5	4	0.8	1.54		41
98	E	21	6	Ozone + Chlorine	200	5	2	0.4	0.39	1.26	41
98	E	21	6	Ozone + Chlorine	2000	5	0	0			41
98	E	21	6	Ozone + Chlorine	20000	5	3	0.6	2.14		41
98	E	21	6	Control	100	5	4	0.8	-0.46	-0.46	41
99	E	21	8	Ozone	1000	5	0	0		2.61	41
99	E	21	8	Ozone	10000	5	1	0.2	2.39		41
99	E	21	8	Ozone	100000	5	3	0.6	2.84		41
99	E	21	8	Ozone + Monochloramine	2500	5	0	0		>3.79	41
99	E	21	8	Ozone + Monochloramine	25000	5	0	0			41
99	E	21	8	Ozone + Monochloramine	250000	5	0	0			41
99	E	21	8	Ozone + Monochloramine	5000	4	0	0		3.79	41
99	E	21	8	Ozone + Monochloramine	50000	4	0	0			41
99	E	21	8	Ozone + Monochloramine	500000	5	2	0.4	3.79		41
99	E	21	8	Monochloramine	50	5	0	0		0.24	41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
99	E	21	8	Monochloramine	500	5	4	0.8	0.24		41
99	E	21	8	Monochloramine	50	5	2	0.4	-0.21	-0.21	41
99	E	21	8	Monochloramine	500	5	5	1			41
99	E	21	8	Control	50	5	1	0.2	0.09	0.31	41
99	E	21	8	Control	500	5	3	0.6	0.54		41
100	E	21	8	Ozone	1000	5	0	0		2.84	41
100	E	21	8	Ozone	10000	5	0	0			41
100	E	21	8	Ozone	100000	5	3	0.6	2.84		41
100	E	21	8	Ozone + Chlorine	500	5	0	0		2.27	41
100	E	21	8	Ozone + Chlorine	5000	4	1	0.25	2.00		41
100	E	21	8	Ozone + Chlorine	50000	5	3	0.6	2.54		41
100	E	21	8	Ozone + Chlorine	2000	5	0	0		3.39	41
100	E	21	8	Ozone + Chlorine	20000	5	0	0			41
100	E	21	8	Ozone + Chlorine	200000	5	2	0.4	3.39		41
100	E	21	8	Chlorine	50	5	2	0.4	-0.21	0.29	41
100	E	21	8	Chlorine	500	5	2	0.4	0.79		41
100	E	21	8	Chlorine	50	5	0	0		0.17	41
100	E	21	8	Chlorine	500	5	5	1			41
100	E	21	8	Control	100	5	2	0.4	0.09	0.09	41
101	E	21	8	Ozone	1000	5	0	0		3.09	41
101	E	21	8	Ozone	10000	5	0	0			41
101	E	21	8	Ozone	100000	5	2	0.4	3.09		41
101	E	21	8	Ozone + Monochloramine	1000	5	0	0		>3.39	41
101	E	21	8	Ozone + Monochloramine	10000	5	0	0			41
101	E	21	8	Ozone + Monochloramine	100000	5	0	0			41
101	E	21	8	Ozone + Monochloramine	5000	5	0	0		>4.09	41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
101	E	21	8	Ozone + Monochloramine	50000	5	0	0			41
101	E	21	8	Ozone + Monochloramine	500000	5	0	0			41
101	E	21	8	Monochloramine	50	5	1	0.2	0.09	0.31	41
101	E	21	8	Monochloramine	500	5	3	0.6	0.54		41
101	E	21	8	Monochloramine	50	5	1	0.2	0.09	0.16	41
101	E	21	8	Monochloramine	500	5	4	0.8	0.24		41
101	E	21	8	Control	50	5	1	0.2	0.09	0.09	41
101	E	21	8	Control	500	5	5	1			41
102	E	1	6	Ozone	100	5	0	0		1.31	41
102	E	1	6	Ozone	1000	5	2	0.4	1.09		41
102	E	1	6	Ozone	10000	5	4	0.8	1.54		41
102	E	1	6	Ozone + Chlorine	300	5	0	0		1.02	41
102	E	1	6	Ozone + Chlorine	3000	5	4	0.8	1.02		41
102	E	1	6	Ozone + Chlorine	30000	5	5	1			41
102	E	1	6	Ozone + Chlorine	500	5	1	0.2	1.09	1.31	41
102	E	1	6	Ozone + Chlorine	5000	5	3	0.6	1.54		41
102	E	1	6	Ozone + Chlorine	50000	5	5	1			41
102	E	1	6	Chlorine	50	5	1	0.2	0.09	0.09	41
102	E	1	6	Chlorine	500	5	5	1			41
102	E	1	6	Chlorine	50	5	1	0.2	0.09	0.16	41
102	E	1	6	Chlorine	500	5	4	0.8	0.24		41
102	E	1	6	Chlorine	50	5	1	0.2	0.09	0.09	41
102	E	1	6	Chlorine	500	5	5	1			41
102	E	1	6	Chlorine	50	5	1	0.2	0.09	0.16	41
102	E	1	6	Chlorine	500	5	4	0.8	0.24		41
102	E	1	6	Control	100	5	4	0.8	-0.46	-0.46	41
103	E	1	6	Ozone	500	5	0	0		2.79	41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
103	E	1	6	Ozone	5000	5	0	0			41
103	E	1	6	Ozone	50000	5	2	0.4	2.79		41
103	E	1	6	Ozone + Monochloramine	2500	5	0	0		3.24	41
103	E	1	6	Ozone + Monochloramine	25000	5	0	0			41
103	E	1	6	Ozone + Monochloramine	250000	5	3	0.6	3.24		41
103	E	1	6	Ozone + Monochloramine	5000	5	0	0		3.54	41
103	E	1	6	Ozone + Monochloramine	50000	5	0	0			41
103	E	1	6	Ozone + Monochloramine	500000	5	3	0.6	3.54		41
103	E	1	6	Monochloramine	50	5	0	0		>1.09	41
103	E	1	6	Monochloramine	500	5	0	0			41
103	E	1	6	Monochloramine	50	5	3	0.6	-0.46	-0.11	41
103	E	1	6	Monochloramine	500	5	4	0.8	0.24		41
103	E	1	6	Monochloramine	50	5	1	0.2	0.09	0.16	41
103	E	1	6	Monochloramine	500	5	4	0.8	0.24		41
103	E	1	6	Monochloramine	50	5	1	0.2	0.09	0.09	41
103	E	1	6	Monochloramine	500	5	5	1			41
103	E	1	6	Control	100	5	3	0.6	-0.16	-0.16	41
104	E	1	8	Ozone	200	5	0	0		1.39	41
104	E	1	8	Ozone	2000	5	2	0.4	1.39		41
104	E	1	8	Ozone	20000	5	5	1			41
104	E	1	8	Ozone + Chlorine	500	5	1	0.2	1.09	1.80	41
104	E	1	8	Ozone + Chlorine	5000	5	1	0.2	2.09		41
104	E	1	8	Ozone + Chlorine	50000	5	4	0.8	2.24		41
104	E	1	8	Ozone + Chlorine	1000	5	0	0		2.47	41
104	E	1	8	Ozone + Chlorine	10000	5	0	0			41
104	E	1	8	Ozone + Chlorine	100000	5	5	1			41

(Continued)

Table B.5 (Continued)

104	E	1	8	Chlorine	50	5	2	0.4	-0.21	0.01	41
104	E	1	8	Chlorine	500	5	4	0.8	0.24		41
104	E	1	8	Chlorine	50	5	0	0		0.79	41
104	E	1	8	Chlorine	500	5	2	0.4	0.79		41
104	E	1	8	Control	50	5	0	0		0.54	41
104	E	1	8	Control	500	5	3	0.6	0.54		41
105	E	21	6	Ozone	500	5	0	0		2.16	41
105	E	21	6	Ozone	5000	5	1	0.2	2.09		41
105	E	21	6	Ozone	50000	5	4	0.8	2.24		41
105	E	21	6	Ozone + Monochloramine	1000	5	0	0		3.39	41
105	E	21	6	Ozone + Monochloramine	10000	5	0	0			41
105	E	21	6	Ozone + Monochloramine	100000	5	1	0.2	3.39		41
105	E	21	6	Ozone + Monochloramine	5000	5	0	0		>4.09	41
105	E	21	6	Ozone + Monochloramine	50000	5	0	0			41
105	E	21	6	Ozone + Monochloramine	500000	5	0	0			41
105	E	21	6	Monochloramine	50	5	1	0.2	0.09	0.16	41
105	E	21	6	Monochloramine	500	5	4	0.8	0.24		41
105	E	21	6	Monochloramine	50	5	1	0.2	0.09	0.44	41
105	E	21	6	Monochloramine	500	5	2	0.4	0.79		41
105	E	21	6	Control	50	5	0	0		0.165	41
105	E	21	6	Control	500	5	5	1			41
106	E	21	8	Ozone	1000	5	0	0		2.54	41
106	E	21	8	Ozone	10000	5	0	0			41
106	E	21	8	Ozone	100000	5	4	0.8	2.54		41
106	E	21	8	Ozone + Monochloramine	5000	5	0	0		3.54	41
106	E	21	8	Ozone + Monochloramine	50000	5	0	0			41
106	E	21	8	Ozone + Monochloramine	500000	5	3	0.6	3.54		41
106	E	21	6	Ozone	1000	5	0	0		2.39	41

(Continued)

Table B.5 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
106	E	21	6	Ozone	10000	5	1	0.2	2.39		41
106	E	21	6	Ozone	100000	5	5	1			41
106	E	21	6	Ozone + Monochloramine	13000	5	0	0		3.65	41
106	E	21	6	Ozone + Monochloramine	130000	5	0	0			41
106	E	21	6	Ozone + Monochloramine	1300000	5	4	0.8	3.65		41
106	E	21	8	Control	50	5	1	0.2	0.09	0.31	41
106	E	21	8	Control	500	5	3	0.6	0.54		41
107	E	21	8	Ozone	1000	5	0	0		1.54	41
107	E	21	8	Ozone	10000	5	4	0.8	1.54		41
107	E	21	8	Ozone	100000	5	5	1			41
107	E	21	8	Ozone + Monochloramine	11000	5	0	0		3.13	41
107	E	21	8	Ozone + Monochloramine	110000	5	2	0.4	3.13		41
107	E	21	8	Ozone + Monochloramine	1100000	5	5	1			41
107	E	21	8	Ozone	1000	5	3	0.6	0.84	1.74	41
107	E	21	8	Ozone	10000	5	3	0.6	1.84		41
107	E	21	8	Ozone	100000	5	4	0.8	2.54		41
107	E	21	8	Ozone + Monochloramine	11000	5	0	0		3.78	41
107	E	21	8	Ozone + Monochloramine	110000	5	1	0.2	3.43		41
107	E	21	8	Ozone + Monochloramine	1100000	5	2	0.4	4.13		41
107	E	21	8	Control	50	5	1	0.2	0.09	0.09	41
107	E	21	8	Control	500	5	5	1			41

Table B.6

Neonatal CD-1 infectivity analysis for experimental trials with natural water F

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
108	F	21	6.3	Ozone	500	5	3	0.60	0.24	0.24	60
108	F	21	6.3	Ozone	5 000	5	5	1.00			60
108	F	21	6.3	Ozone	50 000	5	5	1.00			60
108	F	21	6.3	Ozone + Free Chlorine	500	5	1	0.20	0.89	0.89	60
108	F	21	6.3	Ozone + Free Chlorine	5 000	5	5	1.00			60
108	F	21	6.3	Ozone + Free Chlorine	50 000	5	5	1.00			60
108	F	21	6.3	Ozone + Free Chlorine	1 000	5	1	0.20	1.19	1.69	60
108	F	21	6.3	Ozone + Free Chlorine	10 000	5	1	0.20	2.19		60
108	F	21	6.3	Ozone + Free Chlorine	100 000	5	5	1.00			60
108	F	21	6.3	Chlorine	100	5	4	0.80	-0.81	-0.81	60
108	F	21	6.3	Chlorine	1 000	5	5	1.00			60
108	F	21	6.3	Control	250	5	5	1.00		<-0.41	60
											60
109	F	21	6.3	Ozone	1 000	5	0	0.00		2.19	60
109	F	21	6.3	Ozone	10 000	5	1	0.20	2.19		60
109	F	21	6.3	Ozone	100 000	5	5	1.00			60
109	F	21	6.3	Ozone + Free Chlorine	1 000	5	0	0.00		Spoiled	60
109	F	21	6.3	Ozone + Free Chlorine	10 000	5	0	0.00		Spoiled	60
109	F	21	6.3	Ozone + Free Chlorine	100 000	5	0	0.00		Spoiled	60
109	F	21	6.3	Ozone + Free Chlorine	1 000	5	0	0.00		>3.19	60
109	F	21	6.3	Ozone + Free Chlorine	10 000	5	0	0.00			60
109	F	21	6.3	Ozone + Free Chlorine	100 000	5	0	0.00			60
109	F	21	6.3	Chlorine	300	5	5	1.00		<-0.33	60

(Continued)

Table B.6 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
109	F	21	6.3	Chlorine	3 000	5	5	1.00			60
109	F	21	6.3	Control	200	5	3	0.60	-0.16	-0.16	60
											60
110	F	5	6.3	Ozone	1 000	5	0	0.00		1.87	60
110	F	5	6.3	Ozone	10 000	5	3	0.60	1.54		60
110	F	5	6.3	Ozone	100 000	5	4	0.80	2.19		60
110	F	5	6.3	Ozone + Free Chlorine	1 000	5	0	0.00		2.19	60
110	F	5	6.3	Ozone + Free Chlorine	10 000	5	0	0.00			60
110	F	5	6.3	Ozone + Free Chlorine	100 000	5	4	0.80	2.19		60
110	F	5	6.3	Ozone + Free Chlorine	5 000	5	1	0.20	1.89	2.34	60
110	F	5	6.3	Ozone + Free Chlorine	50 000	5	3	0.60	2.24		60
110	F	5	6.3	Ozone + Free Chlorine	500 000	5	4	0.80	2.89		60
110	F	5	6.3	Chlorine	50	5	1	0.20	-0.11	-0.11	60
110	F	5	6.3	Chlorine	500	5	4	0.80	-0.11		60
110	F	5	6.3	Control	150	5	5	1.00		<-0.63	60
											60
111	F	5	6.3	Ozone	1 000	5	1	0.20	1.19	1.51	60
111	F	5	6.3	Ozone	10 000	5	2	0.40	1.84		60
111	F	5	6.3	Ozone	100 000	5	5	1.00			60
111	F	5	6.3	Ozone + Free Chlorine	500	5	0	0.00		1.89	60
111	F	5	6.3	Ozone + Free Chlorine	5 000	5	1	0.20	1.89		60
111	F	5	6.3	Ozone + Free Chlorine	50 000	5	5	1.00			60
111	F	5	6.3	Ozone + Free Chlorine	1 000	5	1	0.20	1.19	1.69	60
111	F	5	6.3	Ozone + Free Chlorine	10 000	5	0	0.00			60

(Continued)

Table B.6 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
111	F	5	6.3	Ozone + Free Chlorine	100 000	5	4	0.80	2.19		60
111	F	5	6.3	Chlorine	50	5	0	0.00		-0.11	60
111	F	5	6.3	Chlorine	500	5	5	1.00			60
111	F	5	6.3	Control	150	5	3	0.60	-0.28	-0.28	60
											60
112	G	21	5.8	Ozone	1 000	5	0	0.00		3.19	60
112	G	21	5.8	Ozone	10 000	5	0	0.00			60
112	G	21	5.8	Ozone	100 000	5	1	0.20	3.19		60
112	G	21	5.8	Ozone + Free Chlorine	1 000	5	0	0.00		Spoiled	60
112	G	21	5.8	Ozone + Free Chlorine	10 000	5	0	0.00		Spoiled	60
112	G	21	5.8	Ozone + Free Chlorine	100 000	5	0	0.00		Spoiled	60
112	G	21	5.8	Ozone + Free Chlorine	500	5	0	0.00		2.24	60
112	G	21	5.8	Ozone + Free Chlorine	5 000	5	0	0.00			60
112	G	21	5.8	Ozone + Free Chlorine	50 000	5	3	0.60	2.24		60
112	G	21	5.8	Chlorine	50	5	2	0.40	-0.47	-0.47	60
112	G	21	5.8	Chlorine	500	5	5	1.00			60
112	G	21	5.8	Control	50	5	2	0.40	-0.47	-0.47	60
112	G	21	5.8	Control	500	5	5	1.00			60
										0.19	60
113	F	21	6.3	Ozone	1 000	5	4	0.80	0.19		60
113	F	21	6.3	Ozone	10 000	5	5	1.00			60
113	F	21	6.3	Ozone	100 000	5	5	1.00		1.19	60
113	F	21	6.3	Ozone + Free Chlorine	1 000	5	1	0.20	1.19		60

(Continued)

Table B.6 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
113	F	21	6.3	Ozone + Free Chlorine	10 000	5	4	0.80	1.19		60
113	F	21	6.3	Ozone + Free Chlorine	100 000	5	5	1.00		2.14	60
113	F	21	6.3	Ozone + Free Chlorine	2 000	5	0	0.00			60
113	F	21	6.3	Ozone + Free Chlorine	20 000	5	2	0.40	2.14		60
113	F	21	6.3	Ozone + Free Chlorine	200 000	5	5	1.00		-0.11	60
113	F	21	6.3	Chlorine	50	5	1	0.20	-0.11		60
113	F	21	6.3	Chlorine	500	5	5	1.00		<-0.81	60
113	F	21	6.3	Control	100	5	5	1.00			60
										0.87	60
114	F	5	6.3	Ozone	1 000	5	3	0.60	0.54		60
114	F	5	6.3	Ozone	10 000	5	4	0.80	1.19		60
114	F	5	6.3	Ozone	100 000	5	5	1.00		1.69	60
114	F	5	6.3	Ozone + Free Chlorine	1 000	5	1	0.20	1.19		60
114	F	5	6.3	Ozone + Free Chlorine	10 000	5	1	0.20	2.19		60
114	F	5	6.3	Ozone + Free Chlorine	100 000	5	5	1.00		1.86	60
114	F	5	6.3	Ozone + Free Chlorine	1 000	5	1	0.20	1.19		60
114	F	5	6.3	Ozone + Free Chlorine	10 000	5	2	0.40	1.84		60
114	F	5	6.3	Ozone + Free Chlorine	100 000	5	3	0.60	2.54	-0.47	60
114	F	5	6.3	Chlorine	50	5	2	0.40	-0.47		60
114	F	5	6.3	Chlorine	500	5	5	1.00		-0.81	60
114	F	5	6.3	Control	100	5	4	0.80	-0.81		60
										2.19	60
115	G	5	5.8	Ozone	1 000	5	0	0.00			60

Table B.7

Neonatal CD-1 infectivity analysis for experimental trials with natural water G

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
115	G	5	5.8	Ozone	10 000	5	0	0.00			60
115	G	5	5.8	Ozone	100 000	5	4	0.80	2.19	Spoiled	60
115	G	5	5.8	Ozone + Free Chlorine	1 000	5	0	0.00		Spoiled	60
115	G	5	5.8	Ozone + Free Chlorine	10 000	5	0	0.00		Spoiled	60
115	G	5	5.8	Ozone + Free Chlorine	100 000	5	0	0.00		3.53	60
115	G	5	5.8	Ozone + Free Chlorine	5 000	5	0	0.00			60
115	G	5	5.8	Ozone + Free Chlorine	50 000	5	0	0.00			60
115	G	5	5.8	Ozone + Free Chlorine	500 000	5	2	0.40	3.53	-0.11	60
115	G	5	5.8	Chlorine	50	5	1	0.20	-0.11		60
115	G	5	5.8	Chlorine	500	5	4	0.80	-0.11	-1.11	60
115	G	5	5.8	Control	50	5	4	0.80	-1.11		60
115	G	5	5.8	Control	500	5	5	1.00			60
										-0.11	60
116	F	5	6.3	Chlorine	50	5	1	0.20	-0.11	<0.07	60
116	F	5	6.3	Chlorine	75	5	0	0.00		-0.11	60
116	G	5	5.8	Chlorine	50	5	1	0.20	-0.11	<-0.03	60
116	G	5	5.8	Chlorine	60	5	0	0.00		-0.29	60
116	G	5	5.8	Chlorine	75	5	2	0.40	-0.29	-0.11	60
116	F	21	6.3	Chlorine	50	5	1	0.20	-0.11	-0.03	60
116	F	21	6.3	Chlorine	60	5	1	0.20	-0.03	0.07	60
116	F	21	6.3	Chlorine	75	5	1	0.20	0.07	-0.11	60

(Continued)

Table B.7 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
116	G	21	5.8	Chlorine	50	5	1	0.20	-0.11	-0.39	60
116	G	21	5.8	Chlorine	60	5	2	0.40	-0.39	0.07	60
116	G	21	5.8	Chlorine	75	5	1	0.20	0.07	0.09	60
116	G	21	5.8	Chlorine	80	5	1	0.20	0.09	2.21	60
											60
117	G	5	5.8	Ozone	500	5	0	0.00			60
117	G	5	5.8	Ozone	5 000	5	1	0.20	1.89	2.03	60
117	G	5	5.8	Ozone	50 000	5	2	0.40	2.53		60
117	G	5	5.8	Ozone + Free Chlorine	500	5	0	0.00			60
117	G	5	5.8	Ozone + Free Chlorine	5 000	5	2	0.40	1.53	2.53	60
117	G	5	5.8	Ozone + Free Chlorine	50 000	5	2	0.40	2.53		60
117	G	5	5.8	Ozone + Free Chlorine	500	5	0	0.00			60
117	G	5	5.8	Ozone + Free Chlorine	5 000	5	0	0.00		-0.11	60
117	G	5	5.8	Ozone + Free Chlorine	50 000	5	2	0.40	2.53		60
117	G	5	5.8	Chlorine	50	5	1	0.20	-0.11	-0.11	60
117	G	5	5.8	Chlorine	500	5	5	1.00		2.24	60
117	G	5	5.8	Control	50	5	1	0.20	-0.11		60
											60
118	G	5	5.8	Ozone	500	5	0	0.00		2.21	60
118	G	5	5.8	Ozone	5 000	5	0	0.00			60
118	G	5	5.8	Ozone	50 000	5	3	0.60	2.24		60
118	G	5	5.8	Ozone + Free Chlorine	500	5	0	0.00		1.21	60
118	G	5	5.8	Ozone + Free Chlorine	5 000	5	1	0.20	1.89		60
118	G	5	5.8	Ozone + Free Chlorine	50 000	5	2	0.40	2.53		60

(Continued)

Table B.7 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
118	G	5	5.8	Ozone	500	5	1	0.20	0.89	2.49	60
118	G	5	5.8	Ozone	5 000	5	2	0.40	1.53		60
118	G	5	5.8	Ozone	50 000	5	5	1.00			60
118	G	5	5.8	Ozone + Free Chlorine	2 000	5	0	0.00		0.89	60
118	G	5	5.8	Ozone + Free Chlorine	20 000	5	1	0.20	2.49		60
118	G	5	5.8	Ozone + Free Chlorine	200 000	5	4	0.80	2.49		60
										0.72	60
119	G	21	5.8	Ozone	500	5	0	0.00			60
119	G	21	5.8	Ozone	5 000	5	5	1.00			60
119	G	21	5.8	Ozone	50 000	5	5	1.00		-0.11	60
119	G	21	5.8	Ozone + Free Chlorine	1 500	5	3	0.60	0.72		60
119	G	21	5.8	Ozone + Free Chlorine	15 000	5	5	1.00			60
119	G	21	5.8	Ozone + Free Chlorine	150 000	5	5	1.00		1.01	60
119	G	21	5.8	Ozone	500	5	4	0.80	-0.11		60
119	G	21	5.8	Ozone	5 000	5	5	1.00			60
119	G	21	5.8	Ozone	50 000	5	5	1.00		2.89	60
119	G	21	5.8	Ozone + Free Chlorine	1 500	5	2	0.40	1.01		60
119	G	21	5.8	Ozone + Free Chlorine	15 000	5	5	1.00			60
119	G	21	5.8	Ozone + Free Chlorine	150 000	5	5	1.00			60
											60
120	G	21	5.8	Ozone	500	5	4	0.80	-0.11		60
120	G	21	5.8	Ozone	5 000	5	0	0.00		2.89	60
120	G	21	5.8	Ozone	50 000	5	1	0.20	2.89		60
120	G	21	5.8	Ozone + Free Chlorine	1 000	5	4	0.80	0.19		60

(Continued)

Table B.7 (Continued)

Trial	Natural Water	Temperature °C	pH	Treatment	Oocysts Per Animal	Animals in Cohort	Animals Infected	Proportion Infected	Calculated Log Inactivation	Average Log Inactivation	Cyst Batch
120	G	21	5.8	Ozone + Free Chlorine	10 000	5	0	0.00			60
120	G	21	5.8	Ozone + Free Chlorine	100 000	5	1	0.20	3.19		60
120	G	21	5.8	Ozone	500	5	4	0.80	-0.11		60
120	G	21	5.8	Ozone	5 000	5	0	0.00		0.24	60
120	G	21	5.8	Ozone	50 000	5	1	0.20	2.89		60
120	G	21	5.8	Ozone + Free Chlorine	2 000	5	4	0.80	0.49		60
120	G	21	5.8	Ozone + Free Chlorine	20 000	5	1	0.20	2.49	0.89	60
120	G	21	5.8	Ozone + Free Chlorine	200 000	5	1	0.20	3.49		60

APPENDIX C

OZONE PRIMARY TREATMENT INFORMATION FOR EACH SEQUENTIAL TREATMENT TRIAL

Table C.1

Ozone primary treatment information for sequential experimental trials with natural water A

Trial	Oxidant	Applied Dose	Initial Residual C_0	Decay Constant k_d	Contact Time t	Average Ct	Measured Primary Inactivation
		mg/L	mg/L	/min	min	mgxmin/L	log-units
1	Ozone	2.06	1.76	0.34	4.00	3.85	2.17
2	Ozone	2.1	1.48	0.62	4.00	2.19	1.17
3	Ozone	1.96	1.50	0.49	4.00	2.63	1.01
4	Ozone	1.94	1.64	0.37	4.00	3.42	2.17
5	Ozone	1.2	0.77	0.57	2.08	0.94	0.17
6	Ozone	1.22	0.87	0.34	1.87	1.20	0.82
7	Ozone	2.45	2.16	0.56	5.12	3.64	1.68
8	Ozone	2.46	2.22	0.31	5.12	5.70	1.76
9	Ozone	1.48	1.27	0.18	5.02	4.20	2.01
10	Ozone	1.5	1.42	0.12	5.02	5.35	2.36
11	Ozone	0.96	0.83	0.19	1.95	1.35	0.82
12	Ozone	1.47	1.23	0.10	5.08	4.85	2.36
13	Ozone	0.98	0.75	0.12	1.98	1.32	1.32
14	Ozone	1.48	1.42	0.08	5.02	5.87	2.16

Table C.2

Ozone primary treatment information for sequential experimental trials with natural water B

Trial	Oxidant	Applied Dose	Initial Residual C_0	Decay Constant k_d	Contact Time t	Average Ct	Measured Primary Inactivation
		mg/L	mg/L	/min	min	mgxmin/L	log-units
15	Ozone	2.53	1.45	0.37	4.00	3.03	0.81
16	Ozone	2.56	1.53	0.33	4.00	3.40	2.74
17	Ozone	2.38	1.90	0.53	4.00	3.15	1.76
18	Ozone	1.68	1.12	0.59	2.00	1.31	na
19	Ozone	2.31	1.31	0.36	4.00	2.78	2.81
20	Ozone	2.49	1.41	0.39	4.00	2.86	2.74
21	Ozone	2.45	1.55	0.36	4.00	3.29	3.02
22	Ozone	1.7	1.15	0.68	2.00	1.26	0.74
23	Ozone	1.69	0.83	0.63	2.00	0.94	0.67
24	Ozone	1.68	1.18	0.94	2.00	1.06	0.74
25	Ozone	1.7	0.92	0.48	2.00	1.18	0.89
26	Ozone	1.69	1.13	0.43	2.00	1.52	0.89
27	Ozone	3.47	3.46	0.08	20.00	34.52	2.44
28	Ozone	3.53	3.35	0.07	20.00	36.06	2.02
29	Ozone	1.53	1.36	0.16	6.00	5.25	0.31
30	Ozone	1.51	0.96	0.19	6.05	3.45	na
31	Ozone	3.51	2.94	0.11	20.00	23.77	2.22
32	Ozone	1.47	0.82	0.27	6.12	2.46	0.14
33	Ozone	1.47	0.61	0.23	6.00	1.98	0.01
34	Ozone	3.43	2.18	0.04	20.00	30.01	2.42
35	Ozone	3.4	2.34	0.05	20.08	29.65	1.42
36	Ozone	3.66	3.05	0.05	20.00	38.56	2.42

Table C.3

Ozone primary treatment information for sequential experimental trials with natural water C

Trial	Oxidant	Applied Dose	Initial Residual C_0	Decay Constant k_d	Contact Time t	Average Ct	Measured Primary Inactivation
		mg/L	mg/L	/min	min	mgxmin/L	log-units
37	Ozone	3.47	3.01	0.06	20.00	35.06	>3.34
38	Ozone	3.48	3.28	0.08	8.28	19.86	>3.34
39	Ozone	3.51	3.36	0.05	20.00	42.48	>3.34
40	Ozone	1.69	1.25	0.28	4.00	3.01	2.45
40	Ozone	1.08	0.52	0.17	2.00	0.88	1.13
41	Ozone	1.66	1.06	0.17	4.00	3.08	2.42
41	Ozone	1.09	0.48	0.26	2.08	0.77	na
42	Ozone	1.69	1.17	0.45	4.00	2.17	1.77
43	Ozone	1.24	0.69	0.70	2.10	0.76	na
44	Ozone	1.62	0.87	0.70	4.00	1.17	na
45	Ozone	1.68	1.25	0.14	4.00	3.83	1.77
46	Ozone	1.68	1.14	0.36	4.00	2.42	1.42
47	Ozone	1.12	0.61	0.11	2.10	1.14	0.13
48	Ozone	1.07	0.28	0.51	2.38	0.39	0.13
49	Ozone	1.73	1.12	0.20	4.00	3.08	1.77
50	Ozone	1.13	0.50	0.22	2.00	0.81	0.77
51	Ozone	1.65	1.13	0.94	4.00	1.17	1.4
51	Ozone	1.13	0.47	0.96	2.00	0.42	0.96
52	Ozone	1.06	0.57	1.31	2.00	0.40	-0.3
52	Ozone	1.65	0.86	1.40	4.00	0.61	na

Table C.4

Ozone primary treatment information for sequential experimental trials with natural water D

Trial	Oxidant	Applied Dose	Initial Residual C_0	Decay Constant k_d	Contact Time t	Average Ct	Measured Primary Inactivation
		mg/L	mg/L	/min	min	mgxmin/L	log-units
53	Ozone	2.65	0.09	0.59	4.00	0.14	-0.04
53	Ozone	5.68	2.20	1.11	4.00	1.96	1.79
54	Ozone	1.65	0.86	1.40	4.00	0.61	na
55	Ozone	4.6	1.84	1.34	4.00	1.37	1.83
56	Ozone	5.77	2.60	0.76	2.58	2.94	2.57
57	Ozone	4.88	2.84	0.91	1.68	2.44	2.57
58	Ozone	4.86	2.67	0.25	2.80	5.38	<2.57
59	Ozone	3.18	1.08	1.41	2.00	0.72	1.7
60	Ozone	2.92	-	-	1.80	0.00	0.09
60	Ozone	2.92	1.07	2.16	1.50	0.48	0.28
61	Ozone	3.45	1.78	1.78	1.50	0.93	1.4
62	Ozone	3.04	1.40	1.00	1.50	1.09	1.14
63	Ozone	5.47	4.61	0.41	1.50	5.16	0.54
64	Ozone	5.18	3.65	0.52	1.50	3.80	-0.52
65	Ozone	5.25	3.84	0.58	1.63	4.05	-0.52
66	Ozone	2.94	1.50	1.52	1.50	0.89	0.64
67	Ozone	2.86	1.51	2.22	1.50	0.66	1.01
68	Ozone	3.08	1.54	1.77	1.50	0.81	-0.52
69	Ozone	5.4	4.49	0.13	2.40	9.26	-0.16
70	Ozone	5.12	4.18	0.16	2.25	7.90	0.49
71	Ozone	3.7	2.32	0.36	2.67	3.98	2.51
72	Ozone	2.94	2.06	0.44	1.50	2.26	<-0.52
73	Ozone	5.46	4.30	0.31	4.00	9.86	1.14
74	Ozone	3.19	1.80	0.47	2.57	2.69	1.21
75	Ozone	3.06	1.74	0.43	2.50	2.67	2.21
76	Ozone	5.32	4.43	0.16	1.50	5.91	0.84
77	Ozone	5.41	3.98	0.26	4.00	9.90	1.16
78	Ozone	5.34	3.60	0.17	4.00	10.45	-0.16
79	Ozone	4.95	4.20	0.12	1.80	6.80	<-0.46
79	Ozone	5.32	4.78	0.16	1.50	6.37	<-0.46

Table C.5

Ozone primary treatment information for sequential experimental trials with natural water E

Trial	Oxidant	Applied Dose	Initial Residual C_0	Decay Constant k_d	Contact Time t	Average Ct	Measured Primary Inactivation
		mg/L	mg/L	/min	min	mgxmin/L	log-units
80	Ozone	2.98	2.38	0.09	1.75	3.85	1.41
81	Ozone	3.6	3.11	0.17	3.77	8.66	0.01
82	Ozone	3.12	3.61	0.08	5.00	14.88	0.31
83	Ozone	3.27	3.60	0.06	3.00	9.88	0.09
84	Ozone	1.56	1.63	0.08	1.50	2.30	0.99
85	Ozone	3.21	3.55	0.04	1.75	6.00	3.09
86	Ozone	1.53	1.50	0.09	2.75	3.65	1.79
87	Ozone	5.33	5.67	0.02	5.00	26.98	1.29
88	Ozone	3.12	3.27	0.03	3.00	9.38	-0.11
89	Ozone	2.97	3.26	0.09	3.00	8.57	-0.21
90	Ozone	5.33	5.73	0.02	5.00	27.26	1.54
91	Ozone	3.68	4.13	0.07	5.25	18.14	1.69
92	Ozone	3.14	3.44	0.05	3.00	9.58	0.17
93	Ozone	1.45	1.50	0.15	1.50	2.01	1.24
94	Ozone	1.79	2.01	0.22	1.50	2.57	1.74
95	Ozone	3.18	3.09	0.08	1.50	4.37	2.29
96	Ozone	3.09	2.71	0.04	1.00	2.66	1.84
97	Ozone	3.13	3.35	0.03	1.55	5.07	2.46
98	Ozone	3.15	3.69	0.05	3.00	10.28	0.54
99	Ozone	1.52	1.73	0.14	2.50	3.65	2.61
100	Ozone	1.61	1.59	0.13	1.75	2.49	2.84
101	Ozone	1.64	1.38	0.03	2.00	2.68	3.09
102	Ozone	3.11	3.50	0.04	3.00	9.89	1.31
103	Ozone	4.56	5.06	0.02	5.00	24.08	2.79
104	Ozone	3.27	3.50	0.06	5.00	15.12	1.39
105	Ozone	1.61	1.82	0.08	1.55	2.65	2.16
106	Ozone	1.61	1.84	0.15	1.50	2.47	2.54
107	Ozone	1.61	1.76	0.17	1.50	2.33	1.54

Table C.6

Ozone primary treatment information for sequential experimental trials with natural water F

Trial	Oxidant	Applied Dose	Initial Residual C_0	Decay Constant k_d	Contact Time t	Average Ct	Measured Primary Inactivation
		mg/L	mg/L	/min	min	mgxmin/L	log-units
108	Ozone	3.89	3.20	0.99	2.00	2.79	0.24
109	Ozone	3.92	3.85	0.41	2.00	5.25	2.19
110	Ozone	5.37	3.79	0.10	6.00	17.10	1.87
111	Ozone	5.32	3.33	0.09	6.00	15.44	1.51
113	Ozone	3.85	3.30	1.13	2.00	2.62	0.19
114	Ozone	5.41	3.79	0.10	6.00	17.10	0.87

Table C.7

Ozone primary treatment information for sequential experimental trials with natural water G

Trial	Oxidant	Applied Dose	Initial Residual C_0	Decay Constant k_d	Contact Time t	Average Ct	Measured Primary Inactivation
		mg/L	mg/L	/min	min	mgxmin/L	log-units
112	Ozone	1.92	3.32	0.11	2.00	5.96	3.19
115	Ozone	3.69	3.78	0.03	5.00	17.55	2.19
117	Ozone	3.39	3.59	0.04	5.00	16.27	2.21
118	Ozone	3.24	3.30	0.03	5.00	15.32	2.24
	Ozone	3.2	3.46	0.04	5.00	15.68	1.21
119	Ozone	1.01	0.73	0.39	2.00	1.01	0.89
	Ozone	1.02	0.63	0.38	2.00	0.88	-0.11
120	Ozone	1.02	0.71	0.32	2.00	1.05	NA
	Ozone	1.01	0.67	0.38	2.00	0.94	NA

APPENDIX D

SECONDARY TREATMENT INFORMATION FOR EACH SEQUENTIAL TREATMENT TRIAL

Table D.1

Secondary treatment information for sequential trials with natural water A

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
1	Cl_2	4.1	3.4	960	1998	2.82	4.1	2.9	960	1737	-0.18
2	Cl_2	3.6	1.9	960	605	1.82	4.1	2.7	960	1648	0.36
3	Cl_2	1.5	0.4	1000	218	1.59	1.5	0.4	960	219	0.01
4	Cl_2	1.5	0.6	960	305	1.82	1.6	2.7	960	1358	0.17
5	Cl_2	5.2	3.6	940	2303	0.87	5.1	3.8	940	2744	0.01
6	Cl_2	3.2	2.7	970	1760	<0.82	3.0	3.0	960	2415	0.30
7	Cl_2	4.1	3.2	960	1505	2.17	4.1	2.7	960	2176	1.17
8	Cl_2	4.1	3.2	240	728	2.3	4.1	2.9	240	498	0.82
9	Cl_2	4.1	2.3	995	1189	>3.36	4.1	2.3	995	1348	0.82
10	Cl_2	4.1	2.4	995	1382	3.36	4.1	2.2	995	1239	0.82
11	Cl_2	4.2	3.0	1095	1963	2.36	4.2	2.7	1095	1341	1.36
12	Cl_2	3.6	2.4	230	411	2.82	3.6	2.2	230	351	0.59
13	Cl_2	3.6	2.3	235	417	1.17	3.6	2.2	230	418	0.82
14	Cl_2	3.6	2.4	240	435	2.82	3.7	2.4	240	457	0.49

Table D.2

Secondary treatment information for sequential trials with natural water B

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
15	Cl_2	9.8	8.1	960	4282	Na	9.8	8.3	960	5329	0.11
16	Cl_2	6.5	3.3	297	560	>0.81	6.5	3.1	293	630	na
17	Cl_2	9.0	5.5	960	2588	2.74	9.0	5.4	965	3181	-0.19
18	Cl_2	9.3	6.1	1010	2548	1.81	9.3	5.8	1010	2905	-0.19
19	Cl_2	8.1	6.1	950	2660	3.2	8.1	6.6	946	3587	0.51
20	Cl_2	7.4	6.3	180	604	2.81	7.4	6.1	180	632	-0.49
21	Cl_2	9.9	8.5	183	892	3.37	9.9	8.9	185	1013	0.07
22	Cl_2	8.9	8.0	180	834	1.11	8.9	7.8	180	826	0.07
23	Cl_2	8.9	7.4	1005	4717	2.24	8.9	7.3	1010	5051	1.07
24	Cl_2	8.9	8.1	1006	1244	0.81	8.9	7.3	1011	1177	0.31
25	Cl_2	7.1	6.6	988	3802	1.31	7.1	6.5	988	4061	0.37
26	Cl_2	8.9	7.5	182	1107	1.89	8.9	6.6	182	992	0.07
27	Cl_2	7.7	7.1	180	1035	>2.81	8.0	6.8	180	1000	0.63
28	Cl_2	7.9	7.2	180	1141	2.44	7.9	7.0	180	1117	0.23
29	Cl_2	8.2	8.0	187	1335	0.31	8.0	6.9	187	1173	0.01
30	Cl_2	8.0	10.7	119	983	<0.63	8.1	8.2	134	959	0.01
31	Cl_2	5.8	5.6	923	3997	2.51	6.0	5.3	923	4090	0.21
32	Cl_2	6.0	6.3	981	4499	0.14	6.0	5.3	981	3923	0.21
33	Cl_2	6.5	6.3	962	4826	0.92	6.5	6.1	962	4458	0.01
34	Cl_2	5.9	4.2	972	2947	2.31	5.9	4.2	971	2959	0.32
35	Cl_2	7.9	7.2	185	1191	2.21	7.9	7.4	185	1289	-0.16
36	Cl_2	7.9	7.2	888	4936	3.01	7.9	7.2	888	4950	-0.16

Table D.3

Secondary treatment information for sequential trials with natural water C

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
37	Cl_2	6.0	5.9	180	940	>3.64	6.0	5.9	180	957	0.47
38	Cl_2	6.0	4.7	971	3615	>3.64	6.0	4.8	971	3830	0.77
39	Cl_2	6.1	5.9	956	4270	>3.64	6.1	5.9	956	4270	0.47
40	Cl_2	5.7	5.8	976	3922	3.77					
40	Cl_2	5.7	5.9	976	3977	2.13					
41	Cl_2	5.7	5.9	180	920	1.42					
41	Cl_2	5.7	5.9	180	1000	1.42					
42	Cl_2	6.0	6.0	180	951	2.13	6.0	5.8	180	932	0.47
	Cl_2	6.0	6.0	995	3763	2.79	6.0	5.8	995	4150	0.12
43	Cl_2	6.0	6.4	179	1013	>1.01	6.0	5.9	179	934	0.47
	Cl_2	6.0	6.4	1000	4398	>1.01	6.0	5.8	1000	4193	0.47
44	Cl_2	5.9	5.8	947	3981		5.9	5.4	947	3916	na
45	Cl_2	4.9	4.7	184	742	1.95	4.9	4.4	184	707	-0.53
	Cl_2	4.9	4.7	963	3040	>3.77	4.9	4.4	963	3204	0.47
46	Cl_2	6.3	6.3	173	1060	1.47	6.0	5.7	173	873	0.30
	Cl_2	6.3	6.3	976	4268	2.42	6.0	6.0	976	4257	0.47
47	Cl_2	6.3	5.1	171	721	0.77					
	Cl_2	6.3	5.1	973	3274	1.77					
48	Cl_2	6.0	5.9	191	966	1.11	5.6	5.5	195	923	0.3
	Cl_2	6.0	5.9	970	3947	0.77	5.6	5.4	970	3670	0.12

(Continued)

Table D.3 (Continued)

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
49	Cl_2	5.8	5.5	182	829	1.97	5.6	5.3	184	956	<-0.53
	Cl_2	5.8	5.5	987	2926	3.13	5.6	5.4	981	3486	0.83
50	Cl_2	6.0	5.9	186	939	0.77	5.7	5.3	188	854	0.62
	Cl_2	6.0	5.9	972	3649	2.47	5.7	5.2	972	3540	1.47
51	Cl_2	6.9	7.1	225	1017	2.57					
51	Cl_2	7.0	7.2	225	1451	1.07					
	Cl_2	7.0	5.9	934	4575	1.4					
52	Cl_2	6.9	8.7	190	1478	0.23					
	Cl_2	6.9	8.7	984	6187	1.09					
52	Cl_2	6.7	6.2	984	4243	1.36					

Table D.4

Secondary treatment information for sequential trials with natural water D

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
53	Cl_2	8.68	7.36	192	986	0.4					
				967	2931	0.4					
53	Cl_2	7.72	6.21	967	2408	3.4					
54	Cl_2	6.66	6.23	984	4243	1.83					
55	Cl_2	7.56	6.86	201	939	1.4	8.4	7.2	203	1013	0.34
				1009	2841	1.94			1008	3090	0.27
56	Cl_2	8.05	7.09	173	840	2.4	8.4	7.4	235	1073	-0.6
				1013	2945	2.58			1010	3287	0.1
57	Cl_2	6.25	4.74	188	578	2.34	6.9	4.7	191	594	-0.21
				982	996	2.94			982	1351	0.1
58	Cl_2	7.02	5.35	187	615	>3.27	7.7	5.7	190	727	-0.08
				987	1467	3.87			987	1403	0.34
59	Cl_2	7.66	7.26	197	965	2.1	7.8	6.7	194	869	0.1
				992	2904	2.7			992	2942	0.79
60	Cl_2	6.99	8.56	191	1162	0.28					
				954	3879	0.4					
60	Cl_2	7.02	8.35	189	1126	0.83					
				954	3651	1.23					
61	Cl_2	6.85	7.74	188	1023	2.79	7.0	7.8	183	1015	0.27
				1031	1889	2.53			1033	3388	0.53

(Continued)

Table D.4 (Continued)

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
62	Cl_2	6.94	5.94	198	728	1.14	7.0	5.7	200	728	0.37
				960	2419	1.37			960	2307	0.19
63	Cl_2	7.95	7.84	188	1229	<0.17	8.8	8.1	190	1298	-0.16
				988	5814	0.84			988	6038	0.19
64	Cl_2	5.91	6.94	183	1111	-0.16	6.6	7.5	185	1204	-0.46
				955	5038	0.14			955	5452	-0.46
65	Cl_2	5.97	7.23	187	1168	0.48	6.6	7.5	189	1203	-0.46
				989	5153	-0.22			989	5125	0.04
66	NH_2Cl	5.85	8.12	196	1531	2.14	5.9	8.0	198	1538	0.19
				978	7565	>2.51			978	7444	na
67	NH_2Cl	5.82	6.51	196	1239	1.8	5.9	6.4	198	1251	0.68
				272	1747	1.68			272	1733	0.68
68	NH_2Cl	5.97	8.44	134	1080	1.61	6.1	8.3	136	1114	0.51
				254	2064	2.49			252	2027	0.68
69	Cl_2	5.36	6.7	199	1131	0.78	5.9	7.3	201	1270	0.21
				962	4683	1.14			962	5292	0.01
70	Cl_2	5.47	6.91	186	1083	0.79	6.0	7.3	187	1152	0.21
				975	5093	1.31			974	5155	0.01
71	NH_2Cl	5.35	7.83	135	1015	2.54	6.0	8.2	136	1087	0.17
				240	1792	3.14			240	1899	0.37

(Continued)

Table D.4 (Continued)

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	Mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
72	NH_2Cl	5.79	8.31	120	979	<0-0.22	6.2	8.2	122	989	0.21
				246	1995	0.67			246	1983	0.21
73	NH_2Cl	3.95	5.9	130	743	1.19	4.4	6.4	132	828	0.01
				393	2226	2.31			393	2419	0.01
74	NH_2Cl	5.86	7.44	128	922	>3.21	6.0	7.5	130	914	0.21
				248	1754	>3.51			248	1735	0.19
75	NH_2Cl	5.92	9.23	126	1120	>3.21	6.0	8.9	129	1121	0.19
				267	2366	>3.51			267	2334	1.21
76	NH_2Cl	5.77	8.81	122	1030	2.54	6.4	9.2	124	1142	0.37
				244	2096	2.84			244	2247	0.54
77	NH_2Cl	5.38	7.71	131	978	2.84	6.0	8.1	133	1053	0.37
				242	1797	3.51			242	1941	0.84
78	NH_2Cl	5.97	8.69	128	1105	1.09	6.6	9.4	130	1201	0.09
				244	2120	na			244	2256	
79	NH_2Cl	5.56	9.17	123	1121	1.02					
				240	2175	0.24					
79a	NH_2Cl	5.7	9.15	123	1112	0.24					
				240	2144	<0.54					

Table D.5

Secondary treatment information for sequential trials with natural water E

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
80	Cl_2	5.74	7.68	186	1303	1.8	5.9	7.4	188	1295	-0.76
				960	6122	1.84			960	6122	-0.46
81	Cl_2	7.03	5.83	188	930	0.17	7.8	6.3	190	1039	-0.21
				947	3865	0.59			947	4566	-0.76
82	Cl_2	5.96	6.9	186	1248	0.24	6.2	6.0	187	1082	0.09
				973	6397	1.01			973	5517	-0.46
83	NH_2Cl	5.96	6.81	120	817	0.09	6.2	7.2	122	872	
				240	1579	0.54			240	1726	-0.76
84	NH_2Cl	6.11	8.75	123	1070	>2.39	5.9	8.6	125	1057	-0.76
				244	2109	>3.00			245	2072	0.09
85	Cl_2	5.92	6.05	194	1097	2.89	6.2	6.5	195	1183	-0.21
				960	4815	>3.89			960	5166	1.09
86	NH_2Cl	5.94	10.07	130	1301	>3.90	5.7	9.8	135	1311	0.17
				243	2418	>3.91			243	2348	0.79
87	NH_2Cl	5.89	7.51	124	925	1.39					
				240	1781	2.89					
88	Cl_2	5.98	6.27	187	1151	0.09	6.2	6.1	189	1107	0.01
				990	5910	0.46			990	5731	-0.11

(Continued)

Table D.5 (Continued)

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
89	NH_2Cl	5.89	7.82	135	1063	0.84	6.1	8.1	136	1092	-0.26
				241	1862	0.31			241	1938	-0.76
90	NH_2Cl	6.07	7.76	129	1008	2.54	6.7	8.5	130	1102	0.09
				244	1893	>3.69			244	2092	-0.46
91	Cl_2	5.95	6.54	186	1216	1.16	6.2	6.5	188	1214	-0.46
				964	6305	1.89			964	5992	0.24
92	NH_2Cl	5.55	6.6	127	838	0.61	5.7	6.7	129	857	-0.46
				263	1738	0.76			263	1704	na
93	NH_2Cl	5.98	9.02	219	1975	4.39					
94	Cl_2	5.92	8.02	176	1351	2.39	5.8	7.5	178	1280	0.54
				960	6690	2.56			960	6507	-0.11
95	Cl_2	5.69	6.48	180	1156	3.19	5.8	7.9	180	1287	0.01
				960	5932	>4.09			960	6565	1.09
96	NH_2Cl	4.23	5.84	187	1145	4.09	5.2	7.2	187	1345	0.26
97	Cl_2	4.39	4.55	180	750	>3.69	4.4	4.6	950	3441	0.01
				950	3441	4.09					
98	Cl_2	4.84	4.92	240	1153	1.19					
				998	4673	1.26					
99	NH_2Cl	3.89	5.42	92	503	>3.79	3.7	5.5	94	514	0.24
				180	986	3.79			180	979	-0.21

(Continued)

Table D.5 (Continued)

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
100	Cl_2	7.1	5.98	137	781	2.27	6.8	5.7	139	753	0.29
				979	4844	3.39			979	5015	0.17
101	NH_2Cl	3.92	6.03	27	161	>3.39	3.8	5.9	28	164	0.31
				152	901	>4.09			152	885	0.16
102	Cl_2	4.52	3.48	181	602	1.02	4.7	3.6	147	526	0.09
				1062	3320	1.31			183	632	0.16
									1064	3431	0.09
									1116	3581	0.16
103	NH_2Cl	5.13	6.94	113	793	3.24	5.7	6.6	105	697	
				240	1666	3.54			105	697	-0.11
									221	1459	0.16
									240	1584	0.09
104	Cl_2	5.86	6.19	116	702	1.8	6.2	6.3	117	714	0.01
				961	5663	2.47			961	5011	0.79
105	NH_2Cl	6.02	5.83	91	526	3.39	5.8	5.6	92	513	0.16
				150	868	>4.09			150	842	0.44
106	NH_2Cl	3.99	5.83	90	504	3.54					
				150	865	3.65					
107	NH_2Cl	3.96	5.39	90	464	3.13					
				125	689	3.78					

Table D.6

Secondary treatment information for sequential trials with natural water F

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
108	Cl_2	5.2	5.07	0.0136	120.00	299.90	0.84				
108	Cl_2	5.2	5.04	0.0085	220.00	501.55	1.69	5.78	5.66	0.0074	240.00
109	Cl_2	8.37	7.69	0.0019	360.00	2005.09	spoiled	9.32	8.42	0.0020	360.00
109	Cl_2	8.37	7.69	0.0019	388.00	2110.90	>3.19				
110	Cl_2	14.8	13.93	0.0019	60.00	789.92	2.19	16.44	14.88	0.0022	60.00
110	Cl_2	14.8	13.89	0.0011	120.00	1561.48	2.34				
111	Cl_2	14.44	13.80	0.0023	54.00	700.78	1.89				
111	Cl_2	14.44	13.80	0.0013	120.00	1533.29	1.69	16.04	15.14	0.0013	120.00
113	Cl_2	13.28	12.48	0.0036	60.00	673.45	1.19				
113	Cl_2	13.28	12.56	0.0080	125.00	992.43	2.14	13.84	12.56	0.0022	125.00
114	Cl_2	13.93	13.90	0.0023	60.00	779.01	1.69				
114	Cl_2	13.93	13.88	0.0017	120.00	1506.70	1.86	15.48	14.11	0.0013	120.00
116	Cl_2							16.67	13.30	0.0022	60.00
116	Cl_2							16.67	13.29	0.0015	120.00
116	Cl_2							16.67	12.89	0.0035	60.00
116	Cl_2							16.67	12.88	0.0030	90.00
116	Cl_2							16.67	12.86	0.0019	120.00

Table D.7

Secondary treatment information for sequential trials with natural water G

Trial	Type	Sequential Treatment					Secondary Treatment Alone				
		Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation	Applied Dose	Initial Residual	Contact Time	Average *Ct*	Measured Inactivation
		mg/L	mg/L	min	mgxmin/L	log-units	mg/L	mg/L	min	mgxmin/L	log-units
112	Cl_2	14.23	15.67	0.0014	30.00	460.36	spoiled				
112	Cl_2	14.23	15.99	0.0011	47.00	732.43	2.24	13.84	14.57	0.0011	48.00
115	Cl_2	13.75	13.30	0.0009	60.00	776.84	spoiled				
115	Cl_2	13.75	13.30	0.0005	120.00	1549.06	3.53	14.32	13.75	0.0004	120.00
116	Cl_2							16.67	13.57	0.0005	60.00
116	Cl_2							16.67	13.57	0.0003	90.00
116	Cl_2							16.67	13.56	0.0002	120.00
116	Cl_2							15.4	12.54	0.0011	60.00
116	Cl_2							15.4	12.54	0.0001	90.00
116	Cl_2							15.4	12.53	0.0008	105.00
116	Cl_2							15.4	12.54	0.0010	120.00
117	Cl_2	14.39	12.47	0.0006	60.00	734.89	2.03				
117	Cl_2	14.39	12.47	0.0005	70.00	857.80	2.53	14.79	12.54	0.0006	70.00
118	Cl_2	13.72	13.48	0.0006	120.00	1560.74	2.21				
118	Cl_2	13.72	12.88	0.0006	120.00	1491.27	2.49				
119	Cl_2	14.64	12.99	0.0012	60.00	752.00	0.72				
119	Cl_2	14.64	12.35	0.0011	60.00	717.08	1.01				
120	Cl_2	14.64	14.58	0.0013	90.00	1238.34					
120	Cl_2	14.64	13.47	0.0012	90.00	1149.13					

APPENDIX-E

HISTOGRAMS OF MEASURED INFECTIVITY REDUCTION OF *C. PARVUM* OOCYSTS IN THE CONTROL TRIALS FOR THE NATURAL WATERS

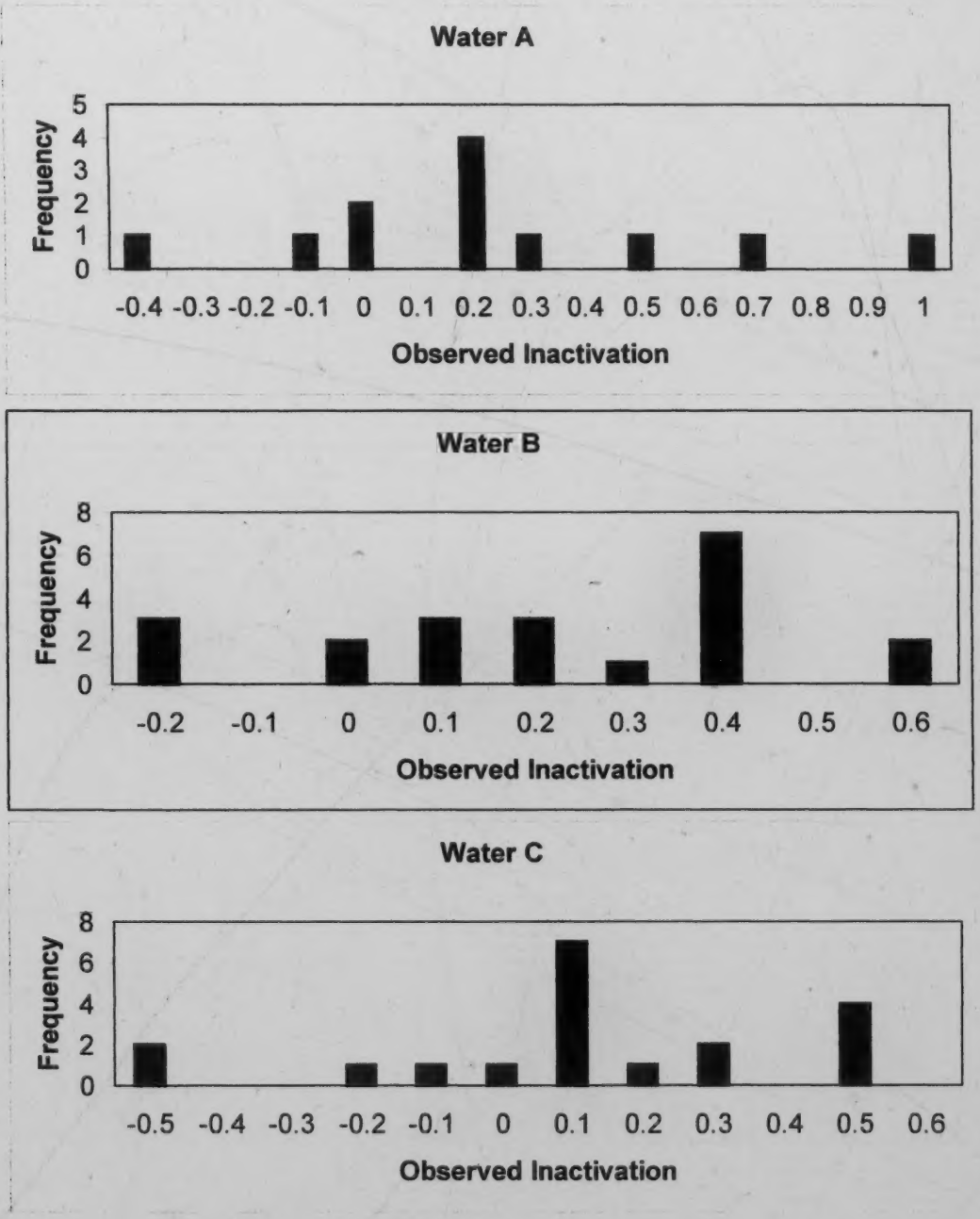

Figure E.1 Histograms of the measured infectivity reduction of *C. parvum* oocysts in the control trials in natural waters A, B, and C.

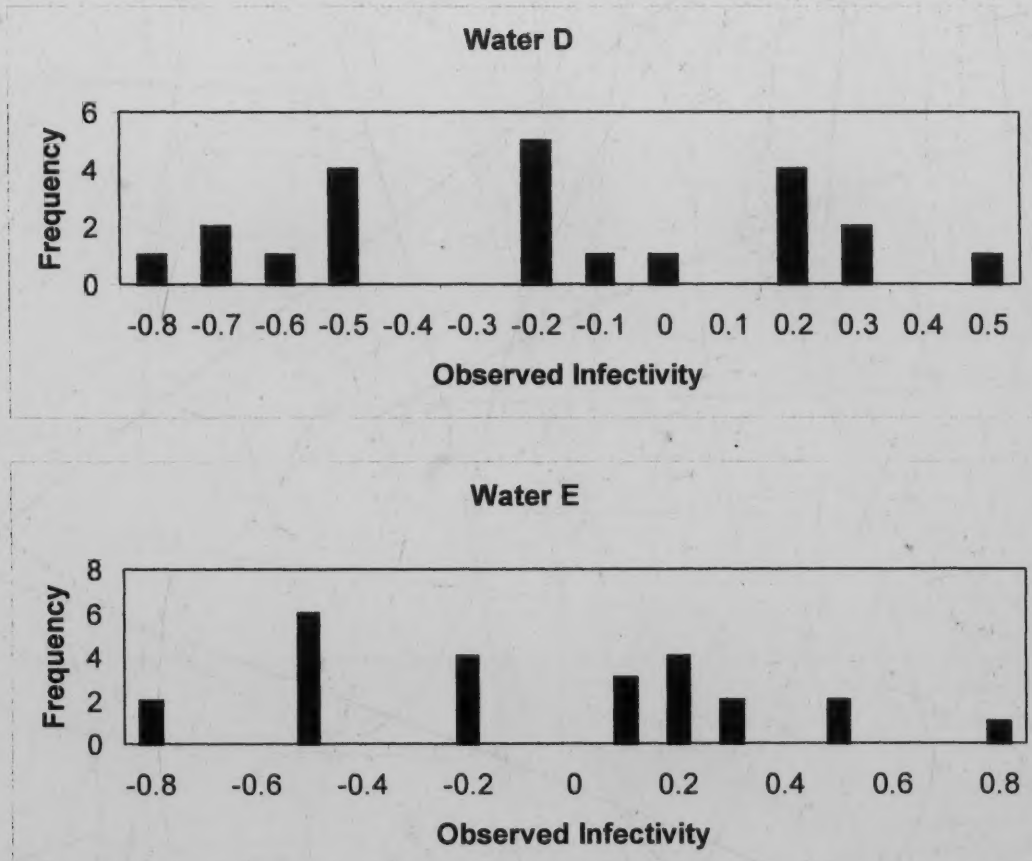

Figure E.2 Histograms of measure infectivity reduction of *C. parvum* oocysts in the control trials for natural waters D and E.

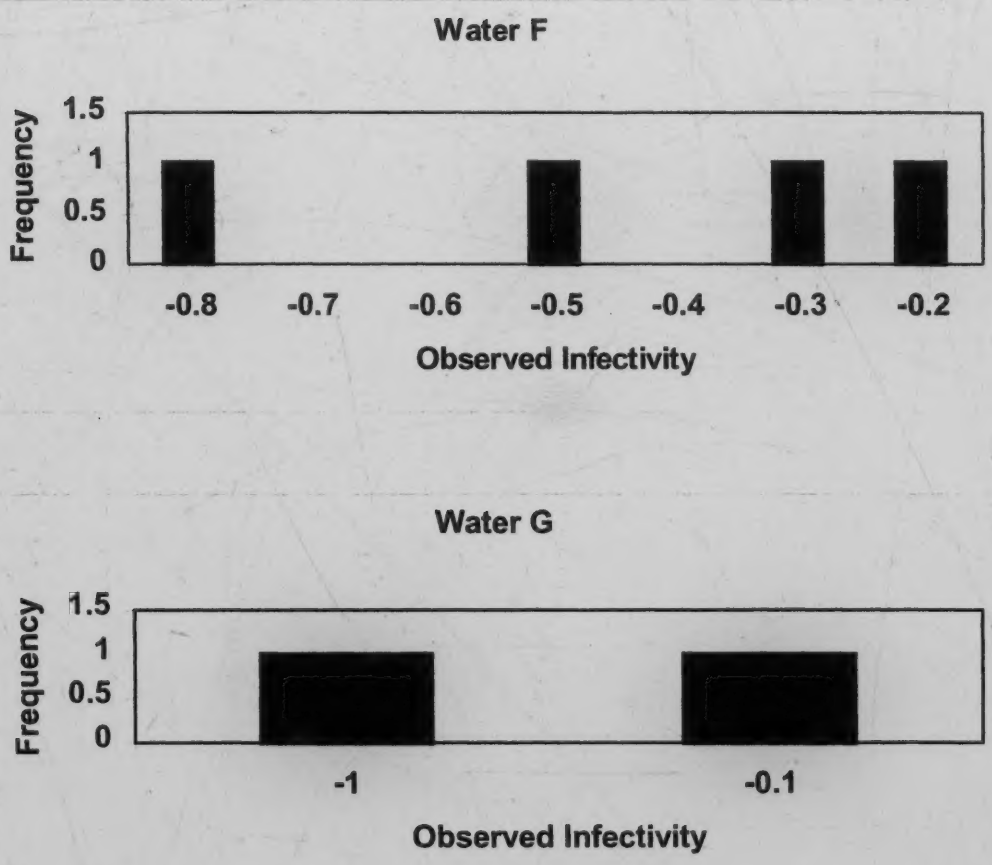

Figure E.3 Histograms of measure infectivity reduction of *C. parvum* oocysts in the control trials for natural waters F and G.

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